

nature*July 17, 1975*

Forever amber on manipulating DNA molecules?

LAST weekend saw a hundred scientists descending on Oxford to discuss with representatives of the Research Councils and the Department of Health and Social Security the problems of experimentation in the 'genetic engineering' field. It is now six months since the Ashby report was published and gave what we described at the time as an amber light to such experiments. The Ashby working party took evidence from more than twenty workers or potential workers in this field, but was itself constituted of scientists who in the main did not include those using the techniques and who might therefore have had a direct interest in the conclusions. Ashby reported that "subject to rigorous safeguards [spelt out in the report] these techniques should continue to be used because of the great benefits to which they may lead". But how to implement these safeguards, and how to implement them quickly?

Several participants were at pains to point out the intellectual pressure that is building up to get moving again in this field. It was wilyly conceded that more impatient scientists were already under way and although there was no evidence that they were conducting experiments in an irresponsible way, their action was causing considerable frustration amongst those waiting for more formal guidance on safety. It is, as one participant put it, rather like the vivisection laws—until society has spoken it is left to the vagaries of individual consciences. And there is obvious pressure to go ahead—participants, on being asked whether they, as individuals, would wish to perform such experiments within the next two years, said 'yes' in considerable numbers.

If the issue were as simple as that, then the most obvious course would be to place as much pressure as possible on the Department of Education and Science, with whom the Ashby report now resides, to get cracking and persuade the government, amidst all its obsessions with consensus in inflation-control to give a moment's thought to the consensus in hazard-control, and implement the safeguards. (The meeting overwhelmingly endorsed the Ashby recommendations on strict containment.) But there are complicating factors.

First, the consensus amongst scientists that all could be made and kept safe is no longer accepted as the watertight guarantee that it once would have been. There has been a certain amount of self-satisfaction that where-

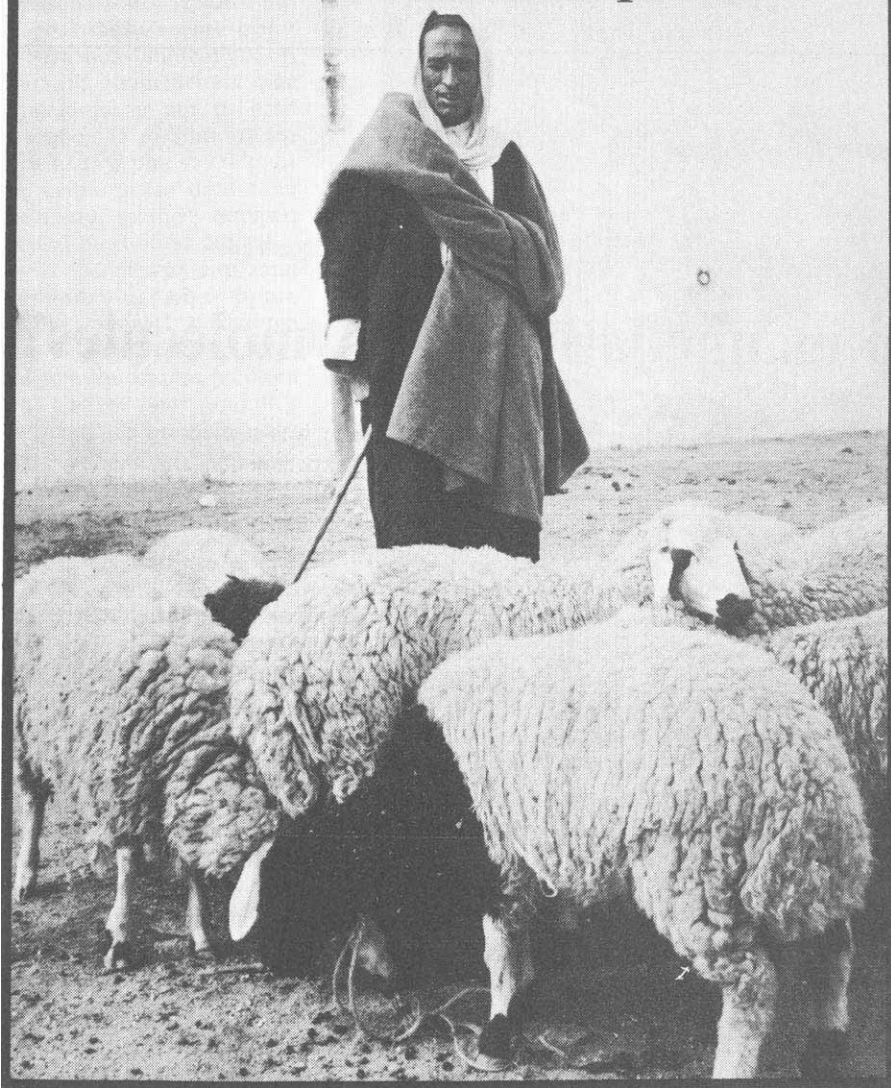
as the Asilomar conference was a meeting of those with a direct interest in the experiments, the Ashby report came from those who could take a broader view. But broad enough? Technicians have an obvious interest in safety, and their concern, expressed through their union, will not necessarily mesh with the scientists' desire to get moving as soon as possible. The pharmaceutical and agricultural industries, barely represented in the Ashby testimony, may at some time in the future wish to switch from laboratory to large-scale operations; they have an obvious interest in ensuring that the safeguards in any legislation make sense on the larger scale. And what about members of the public? Ashby went out of its way to ask 'how can the social values of the community at large be incorporated into decisions on science-policy?', and tried to present the report in a widely-accessible form. But how can the government assess what the community feels on this subject?

Second, there is doubt in some scientists' minds about the scope of any proposed code of practice. There was clear agreement at Oxford that Ashby and Godber should not be confused—that is, that there should be a difference in governmental approach towards the as yet unpredictable hazards of artificial recombinant DNA molecules and the predictable hazards of pathogens with their established handling procedures. But how open-ended should the discussion on unpredictable hazards be left? Some believe that the dangers in currently-known methods may seem slight in comparison with the dangers in methods only dimly foreseen. On the other hand, to widen the debate, the administrators in particular believe, would be to diminish the chances of a quick response from the government.

It may not be an issue of great substance but we feel that the exclusion of the scientific press from the Oxford meeting was unfortunate. We hardly believe that any dark conspiracies were hatched, but there is broader interest amongst scientists in this issue than in almost any other at present, and since one of the purposes of the meeting was to inform and educate, it should be said that an informed and educated press ought to have been one of the (admittedly lesser) aims of the organisers. It is difficult to accept the argument that participants would speak less freely with the press on hand, when there were already a hundred pairs of ears around. □



Man and the Biosphere



When a conscientious government department requests advice on the potential environmental effects of a proposed agricultural development scheme for an area of tropical forest or of the construction of an irrigation dam in an arid area, it is a chastening experience for the "scientist" to have to admit that he has no hard and fast advice to give. Contrary to what most people believe, we neither know for sure what should be done nor, equally important perhaps, what should not be done in these kinds of situations. For, paradoxically, although a great wealth of scientific information is available, much of it is not used and, in fact, in the mono-disciplinary form in which it has been compiled, is unusable within the context of development planning. In spite of the years of work that have gone into the study of forests, deltas and estuaries, arid zone grazing land and irrigation problems, and so on, the findings that have emerged, valuable as they are, do not provide practical answers to the sort of questions governments and decision-makers are asking, because the research approach has not been geared to consideration of the man/environment system involved. It was precisely to resolve this paradox that UNESCO's Man and the Biosphere (MAB) Programme was conceived in an attempt to develop an integrated research approach to the management problems arising from the interactions between human activities and natural systems. Michel Batisse, Director, Department of Environmental Sciences and Natural Resources Research, UNESCO, reports.

THE response to MAB at government level, particularly from the developing countries, was favourable from the start, yet it requires a very long time and much patient effort to get any project moving on a reasonably harmonious front at the international level. The reasons for this inertia are easy to understand. They relate to the traditional suspicion in the older countries of anything 'supranational', to the difficulties of fully conveying the concepts underlying a common objective in a multilingual and multicultural world, and to the mere fact that not all countries are, to put it mildly, at the same stage of development and at the same level of capability. For those with no experience of the intricacies of international cooperation, this slow movement is thoroughly frustrating. For those aware of the problems, it is always a pleasant surprise when some progress is actually made.

The MAB Programme provides a good illustration of this. It originated from the conference in September 1968 on "the scientific basis for rational use and conservation of the resources of the biosphere", which could hardly have dealt with a subject of greater concern to all men and countries. Yet it took some two years before the programme could be actually formulated and officially launched—in November 1970—by the General Conference of UNESCO. And it took another two years of misunderstandings and foot-dragging before it was unanimously endorsed by the Stockholm Conference on the human environment. What had been initiated through the enthusiasm of working scientists had to find its way through the tortuous pathways of politics and the minefields of official science before it could be presented to the world with all the necessary visas and blessings.

This has now been done, and another major step forward has since been achieved; last September, in Washington, DC, the International Coordinating Council which supervises the Programme recognised that the preparatory phase was over and that MAB could now enter its operational phase.

Although MAB was discussed during the summit talks between the USA and the USSR last year—to the great bewilderment of the chancelleries when they discovered this strange acronym in the official communiqué—little publicity has so far been given to the Programme in the scientific press and its actual scientific content is not known to many people potentially interested in it. The general objective has been formulated as follows: "to develop within the natural and social sciences a basis for the rational use and conservation of the resources of the biosphere and for the improvement of the relationship be-

Tunisia: MAB project area No. 3 (grazing lands) examines the problems of rangelands supporting three million sheep units.

tween man and the environment; to predict the consequences of today's actions on tomorrow's world and thereby to increase man's ability to manage efficiently the natural resources of the biosphere".

To achieve this ambitious objective MAB has adopted an integrated, global approach to the analysis of ecological systems, their structure and functioning and their mode of reaction when exposed to human intervention. This approach stresses the impact of man on the environment but also the impact of the environment on man.

The MAB Programme comprises fourteen project areas forming a kind of research matrix in which the main ecological systems and physiographical units (tropical forests, grazing lands, mountains, islands, river basins and estuaries, and so on) interact with major activities or processes such as conservation of genetic resources, use of pesticides and fertilisers, major engineering works or demographic changes.

The MAB Programme is intergovernmental in structure: it is controlled by an International Coordinating Council consisting of representatives of 25 member countries, sitting together with representatives of the organisations of the UN system interested in the Programme (UNEP, the FAO, the WHO, the WMO and UNESCO) and representatives of ICSU and the IUCN. The contribution of each country to the international programme is developed through National Committees, where normally scientists from universities and research institutions sit alongside representatives of government departments. The general secretariat is provided by UNESCO.

Today, after the somewhat tedious but all-important period of preparation during which working groups and expert panels hammered out research guidelines for all projects, the stage is set for actual implementation of co-operative programmes at the regional and international level. Some 80 MAB National Committees have been established, not all of them perfect but most of them keen to move ahead.

Underlying the Programme are three concepts on which its approach to research is based: MAB is problem-oriented, it is interdisciplinary and it aims to provide both immediate and long term scientific, economic and social benefits, in particular to the developing countries.

A problem-oriented approach to research has the twin advantages of projecting the practical face of science to government leaders and of involving decision-makers in the formulation and implementation of projects. Within MAB, the scientist who has traditionally been concerned with the question

"what happens?" in an ecosystem is asked to go a step further and attempt to answer the question on which the decision maker will base his judgement, that is, "what would happen if . . . ?"

Work now being undertaken in Tunisia within the framework of MAB Project Area No. 3 (grazing lands) provides a good example of this problem-oriented approach. The arid and semi-arid areas of southern and central Tunisia cover 65,000 square kilometres and account for some 40% of the national territory. Intense pressure is being exerted on this area by the 3 million "sheep units" it harbours (the various categories of domestic animals being statistically accounted for in terms of sheep equivalents) and by a human population of 1 million urban and 2 million rural inhabitants. The sort of information Tunisian planners want is: what is the carrying capacity of the rangelands and how can it be improved? How far should the development of intensive farming be improved? How far should the development of intensive farming be encouraged? What would be the ecological effects of the introduction of improved fodder crops? The answers provided will have a major bearing on the ecological, economic and social future of the country, and will be of obvious interest to other countries in the Mediterranean area having similar conditions.

The interdisciplinary approach is, by the very nature of things, essential to the MAB programme, but there are considerable difficulties in creating interdisciplinary research teams and in integrating fully the results achieved.

It is easy enough to talk about the need to break down the barriers between disciplines, but many of these divisions have a logical and practical basis. The units of analysis employed often differ from one discipline to another. Socio-cultural studies are usually based on social units—kin groups, tribes, communities—whereas ecological studies may be based on sample areas of a few hectares or even less. Problems of the duration of research also arise. A botanist may be interested in the problem of biomass changes in a plant community during a single growing season, whereas the sociologist may need to examine migration trends over a number of years. If an interdisciplinary effort is to succeed it must be planned as such from the start; each research worker must be prepared to adapt to the methods of others and all must possess the flexibility of mind and the team spirit that this approach implies.

The vital importance of interdisciplinarity is well exemplified in two current MAB projects. In the study on population and environment in the eastern

islands of Fiji (MAB Project Area No. 7—*island ecosystems*) the research team consists of ecologists, pedologists, a nutrition expert, human geographers, a biogeographer and marine biologists. The objective is to understand the actual relationships between population levels and natural resource use under well established conditions. In Hong Kong, where an integrated ecological study of a large urban settlement is being carried out with Australian support (MAB Project Area No. 11—*urban systems*), the spread of disciplines is even wider; the fifty-strong research team ranges from biologists and hydrologists to sanitary engineers, architects and town planners, physicians and psychologists, cultural anthropologists and sociologists. The team also includes a small unit which specialises in the study of the problems of research integration. The objective is, of course, to understand the functioning of the urban system as a particular kind of ecosystem, in order to provide a sounder basis for its planning and management.

It is interesting to note that—in contrast with most previous international research efforts—the MAB Programme stimulates a particularly strong interest in the Third World countries. Determined to shake off the vestiges and constraints of their past, they are eager to exploit their natural resources for their direct benefit. But they are more aware than one thinks of the difficulties involved in changing traditional methods of land use or introducing foreign technologies. They seem therefore to have quickly realised that MAB can offer them tools with which to formulate important aspects of their development planning. A typical example is the problem of the tropical forests. About half the world's forest area is located in three main regions: tropical Africa, tropical America and South-east Asia. Tropical forests are estimated to cover an area of some 2,000 million hectares, of which some 850 million hectares can be regarded as tropical rain forests. Forests are seen by developing countries of the tropics as largely unexploited areas for colonisation and agricultural development as well as a potential source of foreign exchange. For the developed countries they represent a readily available source of relatively cheap timber.

Widespread modification or destruction of tropical forests is of concern to scientists because of their ecological diversity, complexity of structure and richness in species. Within the tropical region, intimate relationships exist between the indigenous populations and the forest, and the forest is important for soil and water conservation. Because of their extent, biomass and dynamics, tropical forests also play an important yet little understood role in the global

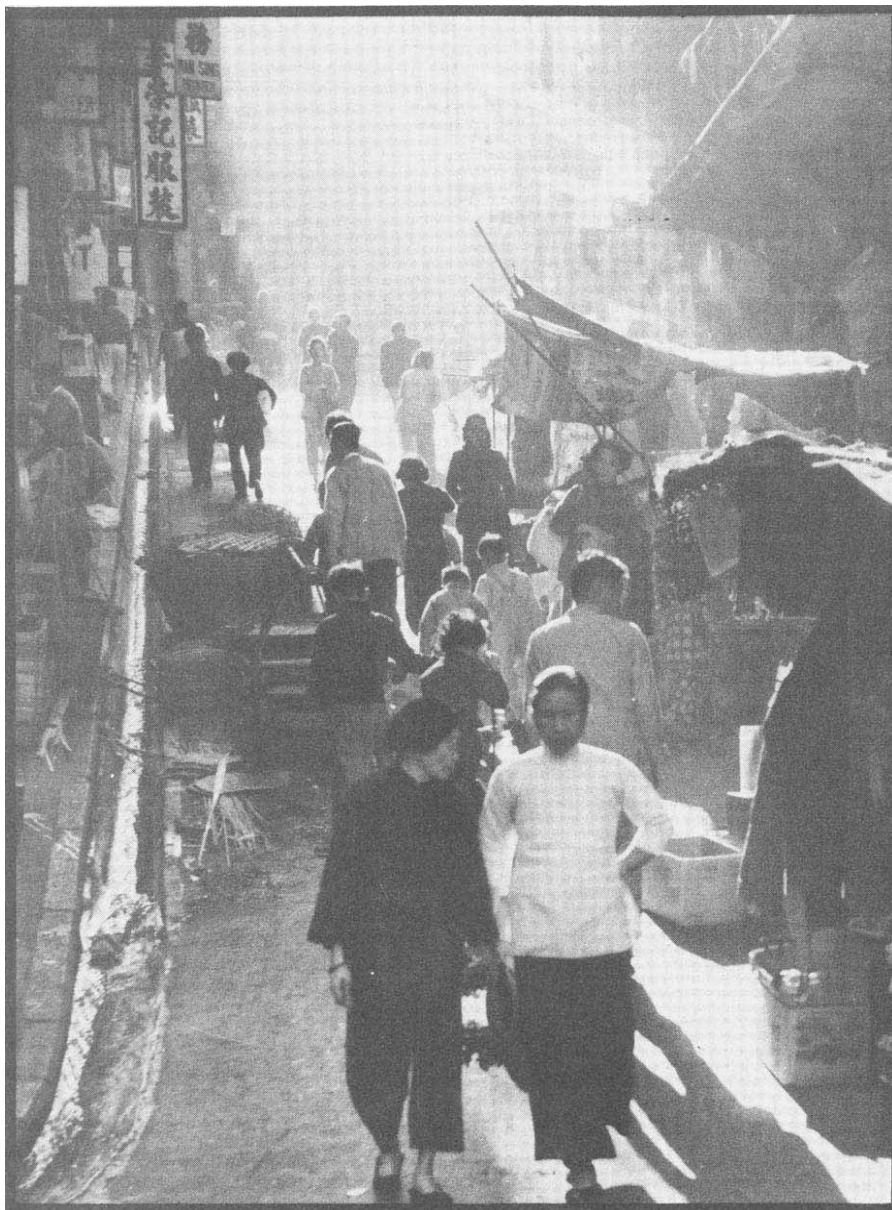
ecological and atmospheric balance of the biosphere.

The importance of research in this field was recognised from the inception of MAB and a general formulation of the practical research problems involved was made in November 1971 under MAB Project Area No. 1 (tropical and sub-tropical forest ecosystems). The next step was to convene in May 1972 an *ad hoc* panel of experts to elaborate the scientific content of research in this area and to consider approaches and plans of studies that could be recommended to the MAB National Committees concerned. This was followed up by an international working group meeting held in Rio de Janeiro in February 1974 which was given the task of identifying problems of common concern and of examining research proposals presented by the different governments that would involve international cooperation. The working group was also asked to discuss the requirements for regional coordination of research, harmonisation of methods and measurements, the exchange of information, experience and personnel, the training of specialists and technicians, and the sharing, storage and processing of data. Finally, regional meetings have been organised in Kuala Lumpur and Mexico City at which national representatives proposed specific pilot field projects for the regions concerned. Similar regional meetings are to be held in Kinshasa and Varanasi later this year.

At the same time regional long term training courses on tropical ecology have been organised in Venezuela, the Philippines and Kenya to contribute to the training of ecologists who could be used in the development of research and management programmes in the three regions concerned. Finally, a world-wide synthesis of knowledge on the tropical forest ecosystems and their rational use and conservation is being prepared for publication in 1976 so as to provide a common background and conceptual basis for MAB Project No. 1.

As a result of this strategy, one can envisage within the near future the development of a network of pilot schemes for integrated ecological research in the three tropical forest regions of the world, involving the exchange of research personnel, providing field training, and forming part of an inter-regional co-ordinated effort to help master one of the most challenging problems of the tropical world. A similar 'strategy' is being applied for other MAB project areas, particularly those on grazing lands and urban systems.

Is MAB now really going to take off? At the last session of the UNESCO General Conference, where some 130



Hong Kong: chosen by MAB for an integrated ecological study.

governments represented were sharply divided on several issues, MAB received unanimous support. The political obstacles therefore seem minimal. Governments, particularly those of the developing countries, are very interested and those scientists already involved in the Programme are committed to its success. The major difficulties are probably elsewhere. They lie primarily in the difficulties—lack of scientific infrastructure and of personnel—developing countries face in playing their role in a world-wide programme, which would be meaningless without their participation. This can only be mitigated through a vigorous training and technical assistance back-up effort in which all donor countries and international organisations concerned should give a hand.

The role already played by UNEP in support of MAB has already been significant, particularly for the regional

activities which are undertaken.

But ultimately the key to the whole operation probably lies in the hands of scientists from the industrialised countries. For them, the programme offers access to areas of the world in which challenging research projects are being developed, either through direct co-operation between MAB National Committees or through multilateral channels. It offers also the interest of adapting to new interdisciplinary methods and to a spectrum of research, the social value of which is beyond question.

Times are difficult everywhere for research funding, but the case for an environmental research programme such as MAB is unanswerable. The scientific community could, if it so wished, demand and obtain the necessary facilities which it alone can mobilise and utilise to make MAB a true and lasting success. □

international news

PRIME Minister Pierre Trudeau has defended Canada's sales of CANDU nuclear reactors to developing countries on the grounds that she cannot withhold from them the advantages of nuclear technology. In an address to the annual meeting of the Canadian Nuclear Association (CNA), he said: "We are a society which has not forgotten its frontier origins. We are a people who have experienced the torment of need, who understand the benefits of sharing. It is inconsistent with that experience and that understanding that we should now deny to the less developed countries of the world the opportunity to gain a handhold on the technological age . . ."

"Surely, if we are ever to eliminate the immense disparities which now separate the living standards of rich and poor, it will be necessary to make available to the disadvantaged every technique at our disposal."

It would be unconscionable to deny to the developing countries the most modern of technologies as assistance in their quest for higher living standards, the Prime Minister said. "But in a world increasingly concerned about depleting reserves of fossil fuels, about food shortages, and about the need to reduce illness, it would be irresponsible as well to withhold the advantages of the nuclear age—of power reactors, agricultural isotopes, cobalt beam therapy units."

As a result of the explosion by India of an underground nuclear device last year, which was made with material from a reactor supplied by Canada, the Canadian government has come under criticism from both within the country and without. Critics have suggested that such an event should have been foreseen by Canada, and prevented.

Since the Indian explosion, the federal Cabinet has done some soul-searching and has tightened its nuclear safeguards policy—but it has also confirmed Canada's intention to go on selling nuclear reactors abroad. This decision, too, has drawn criticism.

In an obvious reference to such criticism, Mr Trudeau said: "We can be proud, as Canadians, of our co-operation with India. The decision taken by Prime Minister St Laurent to enter a nuclear assistance programme with India was a far-sighted and generous act of statesmanship."

Nuclear 'transfers' should always be

Canada answers her nuclear critics

from David Spurgeon, Ottawa

subject to safeguards, however, said the Prime Minister. Canada has now raised the standards of her safeguards "to the point that they are the toughest in the world [and] we refuse to engage in nuclear cooperation without an explicit exclusion of explosive uses."

Mr Trudeau said he did not pretend that the present system of inspection and detection of nuclear cheating was foolproof, and he was "painfully aware that the NPT [Non-Proliferation Treaty] is yet far from universally supported." But he reminded critics that the statute of the International Atomic Energy Agency (of which Canada is a member) charges the agency to spread peaceful applications of nuclear energy throughout the world, "bearing in mind the special needs of the under-developed areas."

(The latest element of the Canadian safeguards policy was announced May 4, 1975, by the Secretary of State for External Affairs at the NPT review Conference in Geneva. It provides that future Canadian bilateral nuclear commitments will be undertaken solely with countries that are parties to the NPT. Also, ratification of the treaty will be an important factor in Canada's decisions on provision of government financing in the nuclear field.)

The existence of some adverse public opinion concerning nuclear matters in Canada was acknowledged at the conference at which the Prime Minister spoke. The CNA's public relations committee said in its report:

"A number of new groups opposed to nuclear power developments have formed in Canada in the past year and there have been modest attempts to form a federation of such groups across the country, which suggests a new phase in the industry's relations with the public may be beginning."

The association's President, J. M. Douglas, President of Babcock and Wilcox Canada Limited, said that although the Canadian government and nuclear industry believe the CANDU system can give great benefit to

Canada through domestic and export markets, "if the opinion of a good majority of Canadians is not in accord with that of government and the utilities, our country's position with regard to the generation of electrical power in a few years' time could be put in jeopardy—as is happening in some other countries."

This seems to be happening because positive, factual information has not been made available in a clearly understood form, Mr Douglas said. "Small, but well organised, environmental groups have used information which is incomplete or inaccurate or misleading, to produce scare stories."

Too often this information—in the absence of factual information supplied from an informed source—has influenced fair-minded people against nuclear power, he said. "We must not let this happen in Canada. And it could. We must greatly increase our efforts to communicate factual information to those who seek it . . ."

Plans already have been made to do this, with a seminar to assist member organisations to communicate with various publics, and a new booklet.

In a review paper, J. S. Foster, President of Atomic Energy of Canada Limited (AECL) said Canada today has 2,500 MWe of nuclear electric generating capacity in operation and a further 6,000 MWe under construction. An additional 7,000 MWe is either committed or at an advanced stage of planning. By the end of the century there will be about 130,000 MWe produced by nuclear plants in Canada.

● L. J. Schofield, of the CNA's Economic Development Committee, says that there is a significant advantage for nuclear power generation in Canada if coal prices escalate at 8% or more a year; such rises would be offset only by sustained capital cost escalation rates in excess of 13% a year. At low coal price escalation rates of 5%, however, the competitive advantage of CANDU stations is small, and could be completely offset by double-digit inflation of capital costs.

"Double-digit inflation," says Schofield, "is a factor to be reckoned with today by the Canadian nuclear industry . . . If the costs of fossil and nuclear stations inflate at equal rates, then the advantage of nuclear generation due to lower fuel costs could be progressively offset by the increasing differential in capital cost." □

Soviet meetings, great and small

from Vera Rich

ONE of the most striking features of Soviet science is the large number of scientific conferences to which it gives rise. Leaving aside, at the one end of the scale, the major conferences of international bodies which happen to have chosen a venue in the Soviet Union and, at the other, small and relatively local gatherings, there still remain 25–30 All-Union meetings, conferences, symposia, and seminars which take place each week. In all, some 1,400 such conferences are scheduled for 1975, with an estimated attendance of some 210,000 scientists and technologists.

Although such conferences are conventionally hymned by Soviet planners as forming one of the most fruitful means of implementing the results of basic research in the national economy and of promoting interdisciplinary studies, in reality not all is well with the Soviet conference scene. According to a recent *Pravda* editorial, many conferences are becoming afflicted with the disease of "gigantomania". With several thousand scheduled speakers, a situation is reached where, even with drastic subdivision into different working groups (which, to a large extent, limits interdisciplinary contacts to outside the lecture hall), each lecturer is allotted only a few minutes in which to deliver only an abstract of his paper. This would be slightly less serious if the audience had already read it in preprint form, so that the short time available could be devoted to questions. But this, too, is not possible—gigantomania besets the preprints too. At the Fourth All-Union Seminar on "Methods and instruments for measuring discharges and quantities of gas" only one-fifth of the papers were ready in preprint form. The post-conference publication of proceedings is likewise extremely tardy so that the papers have lost much of their value before they ever appear in print. This problem, of course, is not peculiar to the Soviet Union—in few other countries, however, would it be considered so urgent as to merit editorial comment in the country's leading newspaper, recommending that steps should be taken to rectify the situation by all the bodies responsible, from the Academy of Sciences and the relevant ministries downwards.

● The overlap between the two forms of Soviet scientific meetings—the vast gigantomaniac official gathering and the small illicit gathering of refusniks—sometimes approach each other in a manner somewhat perturbing to the

authorities. This, according to his sons, is one of the main reasons why Academician Veniamin Levich has received such opposition to his desire to emigrate to Israel. So far, he is the only Member of the Academy of Sciences to request a visa (Professor Tumermann was, at the time he applied for a visa working at the Lebedev Physics Institute of the Academy but



Academician Levich: promised a visa.

was not himself an Academician). Levich who has long since been dismissed from his post still remains a member of the Academy, and, technically speaking, still retains the right to attend meetings. Although Levich's works are no longer mentioned by establishment Soviet scientists, and references to them in incoming Western works are blotted out by the censor, the authorities seem to prefer to let him remain an Academician, rather than put into action the complex machinery of expulsion, which could well bring a backlash of unfavourable publicity. Academician Levich has been promised his exit visa—"by the end of 1975". With the 250th anniversary celebrations of the Academy scheduled for October, it will be interesting to see whether Academician Levich will receive his visa before that date—or if not, whether he will assert his right to participate in the jubilee meeting.

● Another interesting problem is that of refusnik scientists invited to take part in international conferences to be held within the Soviet Union. The case of cyberneticists Aleksandr Lerner and Evi Gel'man is likely to provoke a problem for the Soviet authorities.

Lerner and Gel'man have been invited by the international organising committee to take part in the Conference on Artificial Intelligence to be held in Tbilisi this September. Judging from the case of Zhores Medvedev, who was forcibly prevented from attending the Kiev Conference on genetics, it seems unlikely that Lerner and Gel'man will be permitted to attend the Tbilisi Conference. How the problem will be resolved is still a matter for speculation, but latest reports indicate that Gel'man has been promised an exit visa, although he has not, to date, actually received it. To send both of them abroad before the Conference would certainly prove the happiest solution for the two refusniks themselves, and could well be the easiest way out for the Soviet authorities of what might otherwise prove an embarrassing and publicity provoking situation.

● A conference liable to cause a certain unease to the Soviet authorities was that of the Royal College of Psychiatrists, which held its annual meeting in London last week. The meeting unanimously confirmed a decision to send a telegram of protest to Professor A. V. Snezhnevskii, President of the Institute of Psychiatry of the USSR, deploring the misuse of psychiatry as a means of political repression. The telegram, signed by Sir Martin Roth, president of the college, cites specifically, "the continued incarceration of Gluzman, Bukovskii and Blyushch, which appears a perversion of psychiatric practice and the denial of natural justice".

The case of Dr Gluzman, whose incarceration in a mental hospital seems to have been the direct result of his preparation of a report on psychiatric malpractice in the case of General Grigorenko, was raised at last year's Annual Meeting. Since then Sir Martin Roth has sent two letters to Professor Snezhnevskii on Gluzman's behalf, the first of which received an extremely unsatisfactory answer, stating that the case against Dr Gluzman contained no references to the Grigorenko affair. The second letter, which in effect was an enquiry as to what then were the charges against him, received no answer.

The telegram of protest also cites the case of Vladimir Bukovskii, co-author with Dr Gluzman of the famous *samizdat* "Manual of Psychiatry for Dissidents" (a guide to what to do if accused of mental illness as a result of one's political views) and Leonid Plyushch, the Ukrainian mathematical, who, as a result of the "treatment" he has undergone since hospitalisation for dissidence is now, according to the latest report (from an informant who saw him last April) in a state of virtual

catatonia with probably irreversible mental damage.

In addition to the protest to Professor Snezhnevskii, the meeting also resolved to send a telegram to Dr Gluzman himself—the first time that this particular form of protest has ever been undertaken by the college.

Among those who spoke in favour of this action was Dr Denis Leigh, Secretary General of the World Psychiatric Association, who said that in his view the type of action envisaged by the college was welcome and much to be encouraged. □

Oxford man wins large prize

PROFESSOR X has won for Dr Y from Oxford (who prefers to remain anonymous) a year's free subscription to *Nature*. Professor X is on 12 editorial boards and easily heads the list in *Nature's* competition (May 8); the runner-up could muster a mere nine appearances. □

THE effects of inflation on the prices of technical and scientific books are illustrated in this table of findings from a British survey. Eleven publishers submitted information for the survey, which was carried out by the Technical and Scientific Group of the Publishers Association. Each was asked to give the extent and price, on publication, of their new scientific and technical books (and new editions) published in the years 1972, 1973 and 1974. Where there were two editions

(eg, cased and paper) the cheaper was taken. In the case of 1974 they were asked to distinguish between publication in the first and second halves of the year. They were also asked to assign each book to a category, namely: A: books intended for purchase by undergraduate students; B: books intended for purchase by senior students, lecturers, practitioners, libraries, etc; C: books for which only a library sale is expected. The most rapidly rising cost was that of paper.

Year	Size of Sample	Total no. of pages	Total price (£)	Price/100pp (£)	% above 1972	
1972	157	44462	381.75	0.86	—	Category A
1973	140	39062	390.15	1.00	16.2	
1974(1)	74	21343	218.25	1.02	18.6	
1974(2)	49	12029	138.75	1.15	33.7	
1972	100	29297	457.95	1.56	—	Category B
1973	95	28831	483.53	1.68	7.7	
1974(1)	57	18511	333.95	1.80	15.4	
1974(2)	35	9657	179.70	1.86	19.2	
1972	31	12169	249.20	2.04	—	Category C
1973	45	18842	397.00	2.11	3.4	
1974(1)	23	8696	231.50	2.66	30.4	
1974(2)	10	4073	163.80	4.02	97.0	

correspondence

Deep in Egypt

SIR,—Having carried out surveys during and after the Second World War in Libya, Egypt and the Sudan, I was very interested to read the article (June 19) on the project for producing power by diverting Mediterranean water into the Qattara Depression by a canal through the Alamein area. Your correspondent was incorrect, however, in ascribing the initial surveys and ideas for this project to Professor Bassler, an ex-officer of Rommel's army; it is much older than that. In fact it dates back to the previous war, since it was in 1917 that Dr John Ball, the Director of the Egyptian Desert Survey, lent a small aneroid barometer to the officer in charge of a British Light Car Patrol who was going to travel in the area. The officer came back without the aneroid but with a reading for the Qattara Spring of some 60 metres below sea level—and presumably with a somewhat tarnished reputation as a surveyor!

Dr Ball did not, however, forget this odd result; and in 1926 he was able to send a professional surveyor, G. F. Walpole, to make a more detailed investigation, which revealed for the

first time the full extent and depth of this remarkable feature. Describing this and other investigations in 1927 in the *Geographical Journal*, Dr Ball then suggested that the depression might be used as a source of power by diverting Mediterranean water into it through tunnels. Six years later, when a complete topographical and geological survey had been made, he published a long paper in the same journal, giving detailed estimates of the sizes of tunnels required, the energy available and the length of time (several hundred years) that the project could last. Clearly the rate at which salt will accumulate even from sea water is very much slower than the rising tide of silt in the upper reaches of Lake Nasser, even though with modern engineering techniques much larger channels or tunnels can be envisaged.

Dr Ball, like Dr H. E. Hurst who organised the comprehensive collection of data about the Nile Basin on which so many projects were based, belonged to a small band of British surveyors and scientists who worked for many years in Egypt in the early years of this century. With the wisdom gained from long experience in the area added to their native wit and scientific training, they

wrote a number of far-sighted papers which those who are now suggesting new versions of their projects will do well to study.

Yours faithfully,

JOHN WRIGHT

Effingham, Surrey

English and editorial boards

SIR,—Mr Andrewes describes himself as a hack. Nothing in the wording of his letter (July 10) suggests that the epithet is justified, but there are many people around editorial offices to whom it is applicable. Instead of confining themselves to turning poor into passable English, they seem to delight in trying to turn straightforward English into jargon. In a proof that came last week an editor was trying to get me pompously to refer to myself as 'the author'. Proofs of two papers sent to a microbiological journal a few years ago had been so mangled that I withdrew them and published them as originally written in *Proc. R. Soc. B*. A friend had a recent paper to a nutritional journal turned in places into germanic near gibberish. And so it goes on, making people who recognise clear English think that all scientists write badly.

If a paper comes within the province of a journal and seems to be scientifically acceptable, an editor's job is the elimination of ambiguity and prolixity. These are matters of fact. An editor can say: "This phrase could mean A or B. Which do you mean?" or "This amounts to so-and-so, which is only half as long as the original." It is no part of an editor's job to insert misconceptions about the English language, however sincerely felt, into someone else's paper.

Yours faithfully,

N. W. PIRIE

Harpenden, Herts,

Fostering spin-off

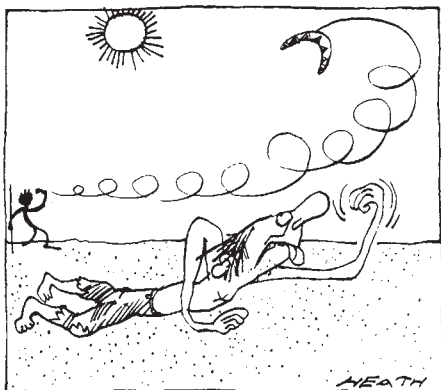
SIR,—It was with much interest that I read the recent paper by Isaacs *et al.* (January 24) on cyclogenesis by motor vehicle movements. I wonder how many people outside Australia are aware that the Aborigines have been making use of this principle for centuries. Why else would their rain making dances consist of a steady clockwise progression?

Related to this, I am at present working on a theory that the drying up of central Australia followed the invention of the boomerang, whose anticlockwise rotation would inhibit cyclonic activity. As happens with most revolutionary ideas, however, I have been having trouble funding this programme.

Yours faithfully,

K. H. LLOYD

Salisbury Heights, South Australia.



Darwin's cancer research

SIR,—In considering the scope of subject matter relevant to the study of cancer, Stoker (April 17) suggests that much of cell-oriented biology may have such implications. It may, but it would be shortsighted to stop there. Natural selection has obvious relevance, at least obvious to an evolutionary biologist, and Cairns (May 15) has discussed this explicitly. It has also become central to immunology. My only contribution to oncology came as a direct result of work in palaeoecology. Theoretical frameworks that develop in one subject often have application elsewhere, and this really cannot be foreseen. Would

the Imperial Cancer Research Fund have supported Darwin?

Yours faithfully,

LEIGH VAN VALEN

*University of Chicago,
Chicago, Ill. 60637*

Integrity in science

SIR,—I am sorry that the only comment accompanying my letter (June 12) mainly concerns its occasion and not its essence. I would gladly ignore it, but the claims of truth preclude that luxury.

Mr. Maddox's suggestion that the non-appearance of his leader resulted from the "offensiveness and repugnance" of the correspondence is false. The article was promised, "before the end of the year", on November 24, 1969. Successive (polite) enquiries from Lord Soper and me brought such replies as "the article is now almost ready" (January 21, 1970); it would be delayed "a week or two" (end of March 1970); until Lord Soper's final inquiry (July 6, 1970) brought no reply—or the article. It had been promised in place of my reply to Professor Synge who had written (*Nature*, 219, 790; 1968) that "as the result of a lengthy correspondence with Professor Dingle" he and I had agreed that either "the concepts used in the special theory of relativity as ordinarily understood" or "the concept of clocks that run regularly, as understood by Professor Dingle" must be abandoned. Since my concept of such clocks has never been represented as other than an instrument so recognised by a standard observatory, the only relevant property I required being an inability of one to run concurrently both faster and slower than another, it was now clearly mate in one move. Maddox made that move impossible by steadfastly suppressing my rejoinder; it has never appeared. (Incidentally, his statement now that I have "shifted my ground" since 1968 is here seen clearly to be false: Synge's diagnosis is identical with my position as stated in my recent letter).

Maddox should have quoted more of my letter of April 6, 1971; it ran: "In view of the failure of all other means of getting a straightforward answer to my criticism of special relativity . . . I have been, with great reluctance, forced to the extreme measure of writing a book describing the course of the whole series of evasions . . . I write this final letter, which will be included in the book if necessary, to invite you to give your own explanation of your attitude, which I promise to include verbatim. I repeat what I have said before—that my sole object is to get this matter settled, first of all with absolute openness and avoidance of all further evasion and quibbling, and secondly,

provided that that is done, with the minimum of sensation and unpleasantness. Action on your part, even now, would make a full exposure of the ethical aspect of the matter unnecessary". Your readers can judge whether the "offensiveness and repugnance" of this letter adequately excuse the succession of broken promises a year earlier.

There was a sequel to "the recent incident" as related by Mr. Maddox. The author of the article mentioned subsequently asked for his side of the story (although, since it had already appeared in the Editorial, this might well seem redundant) and was given a vague statement that implied, if anything, that the "promises" ascribed to me had another source than that which he now owns, which was "even more full of somewhat unrealistic threats". I know of no letter which can be so interpreted. It was to prevent a recurrence of such misrepresentation and for that reason alone that I felt it necessary to record the actual facts.

I suppose I must reply once more to the charge that "Dingle's confusion stems from his assertion that special relativity requires that the differences of rate should 'actually and not merely apparently' occur. The truth, of course, is quite the opposite." Whenever the special relativity effects of motion are invoked to predict or explain something observable (for example, asymmetrical ageing, cosmic-ray behaviour . . .) they are held to be "actual", whenever this leads to a contradiction they become only "apparent"; and anyone to whom this is unacceptable is deceived by "commonsense". The letter I have called "L" completely refutes Maddox's statement. His technical example involving "lasers" and "algorithms" refers to a completely different phenomenon which has nothing at all to do with the matter.

I withhold comment on the rest of Maddox's letter, remembering what Shakespeare said about painting the lily, and leave your readers to judge whether Maddox is right in denying that there is "an ethical issue" here. I am sure, however, that the many who agree with me that there is, will require a plain, direct answer to my question if they are to retain their trust in the integrity for which the scientific world has in the past been justly noted. I most earnestly hope that among those with authority and responsibility in this matter, there will not be wanting someone ready to have done with "double-thinking", to clear his mind and words of cant, and to exhibit the candour and courage needed to provide such an answer.

Yours faithfully,

HERBERT DINGLE

Purley, Surrey

news and views

Applying molecular genetics to a human disease

from Edward J. Benz and David G. Nathan

THE α - and β -thalassaemia syndromes are a heterogeneous group of inherited haemoglobinopathies characterised by reduced or absent synthesis of structurally normal α or β globin chains. The first step to identifying the nature of the syndromes was made in 1971 when the *in vivo* defect in chain synthesis was reproduced *in vitro* using β -thalassaemia globin mRNA (Nienhuis and Anderson, *J. clin. Invest.*, **50**, 2458; Benz and Forget, *J. clin. Invest.*, **50**, 2755). Then a quantitative deficiency in globin mRNA was demonstrated by the technique of hybridising thalassaemic globin mRNA with radioactive DNA complementary to globin mRNA (Housman *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1809; 1973; Kacian *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1886; 1973). Total absence of cytoplasmic β chain mRNA in homozygous β^0 -thalassaemia (Forget *et al.*, *Nature*, **247**, 379; 1974; Benz *et al.*, *Blood*, **45**, 1; 1975) and α -chain mRNA in homozygous α^0 -thalassaemia (Kan *et al.*, *J. clin. Invest.*, **53**, 37a; 1974) has subsequently been demonstrated both by translation in cell-free systems and by complementary (c) DNA-mRNA hybridisation.

It is, therefore, now clear that the primary genetic lesion in thalassaemia is a defect in the synthesis, processing, transport, or stability of the α chain mRNA (in α -thalassaemia) or β -chain mRNA (in β -thalassaemia). Since total absence of mRNA in the α^0 - or β^0 -thalassaemias suggests gene deletion, investigators are now concentrating on the regulation of human globin gene expression. These disorders thus continue to be the examples *par excellence* of human diseases to which the techniques and concepts of molecular biology can fruitfully be applied.

Ottolenghi *et al.*, (*Nature*, **251**, 389; 1974) and Taylor *et al.*, (*Nature*, **251**, 392; 1974) recently isolated total cellular DNA from livers of infants with Hb Bart's hydrops foetalis, the homozygous form of α^0 -thalassaemia. This DNA hybridised to purified β -chain cDNA as efficiently as normal DNA but failed to form any double stranded hybrids with α -chain cDNA. This constitutes strong evidence that these infants had no α chain genes

and that, therefore, the α^0 -thalassaemia allele is a gene deletion.

Kan *et al.* (*Nature*, **255**, 255; 1975) have now applied the same approach to answer a more vexing question: the number of α chain genes present in normal man. There has been considerable controversy over whether the haploid number of chromosomes contains a single α chain gene or duplicated α chain genes (Lehmann and Carrell, *Br. Med. J.*, **4**, 748; 1968; Koler and Rigas, *Ann. hum. Genet.*, **25**, 95; 1961; Hollan *et al.*, *Nature*, **235**, 47, 1972; Abramson *et al.*, *Science*, **169**, 194; 1970; Milner *et al.*, *Lancet*, **i**, 729; 1971). The two hypotheses make very different predictions about the genetic basis of the four classical α -thalassaemia syndromes described in the Asian populations among whom α -thalassaemia is most common. These syndromes include an asymptomatic silent carrier state (α -thalassaemia₂ trait), a mild hypochromic anaemia (α -thalassaemia₁ trait), a moderately severe haemolytic anaemia (Hb H disease), and fatal hydrops foetalis; the deficits in α chain synthesis in each syndrome correspond well to the clinical severity of each disorder. The 'one gene' theory postulates the existence of two forms of α -thalassaemia gene: a 'mild' (α^+ -thalassaemia) allele, and a 'severe' (α^0 -thalassaemia) allele heritable at a single locus. The four syndromes would then result from various combinations of normal, α^+ , and α^0 alleles in the diploid state. The two-gene theory postulates that all α -thalassaemia alleles are α^0 -thalassaemia alleles. In increasing order of severity, the four syndromes would then result from inheritance of α^0 -thalassaemia alleles at one, two, three or all four of the α chain loci available in a diploid cell containing duplicated α -chain genes.

Since all α chain genes are deleted in hydrops foetalis, the α^0 -thalassaemia allele can be regarded as a gene deletion regardless of which model, one gene or two gene theory, is correct. But the one-gene model predicts that a patient with Hb H disease (α^+ -thal/ α^0 -thal) would have half the normal number of α chain genes. The two-gene model for Hb H disease (α^0 -thal/ α^0 -thal/ α^0 -thal- α) predicts that this

patient would have only one quarter the normal number of α -chain genes.

Kan *et al.* have now compared the different rates at which DNA from normal and Hb H disease patients hybridises to fixed amounts of α chain cDNA. This method of kinetic analysis depends on the fact that formation of double-stranded hybrids between a fixed-amount cDNA base sequence and a homologous base sequence in cellular DNA will depend on the concentration of the cellular DNA sequence (in this case, an α -chain gene) and time. By measuring the percentage of the cDNA input bound into hybrids at various times, one can construct a 'C₀t' curve (C₀t=initial concentration of cellular α chain DNA sequences $\times$ time of reaction); plots of percentage hybridisation against the C₀t values at each time point permit comparison of the relative rates at which cellular DNA from each type of patient hybridises to the α chain cDNA. The C₀t_{1/2} (C₀t value at which 50% hybridisation occurs) of each curve then serves as a measure of the α chain gene content of one DNA sample relative to the others. When these investigators compared normal DNA to Hb H disease DNA, the C₀t_{1/2} for the Hb H sample was roughly four times as high as that for the normal sample implying that the Hb H sample had only one quarter the normal complement of α chain genes.

These data seem to provide direct biochemical evidence supporting the two-gene theory for α -thalassaemia and the existence of duplicated α chain alleles in normal man. But the use of C₀t curve analysis is fraught with technical difficulties when applied to the analysis of genes present in one or only a small number of copies in human DNA. The α chain genes account for only 10⁻⁷-10⁻⁸ of the total base sequences present in the human genome. Hybridisation between α -chain cDNA and these α chain genes thus occurs very slowly, necessitating the plotting of the C₀t values on a logarithmic scale. Two- to four-fold differences are relatively difficult to measure from the plotted curves. Kan *et al.* devised a set of experiments to determine whether their method could accurately

define this range of differences in $C_{0t_{1/2}}$. By mixing hydrops foetalis DNA (no α chain genes) with normal DNA in known proportions, they created artificial mixtures having a quarter and half the normal concentration of α chain sequences. The quarter mixture gave $C_{0t_{1/2}}$ values with α chain cDNA identical to those obtained with Hb H disease DNA. These results support the technical validity of the $C_{0t_{1/2}}$ values measured for the Hb H DNA samples.

The application of the same approach to β^0 -thalassaemia would seem to be straightforward. Unfortunately, the δ chain of Hb A₂ ($\alpha_2\delta_2$) is structurally very similar to the β chain (only 10/146 amino acid differences). Forget *et al.* (Nature, 247, 379; 1974) have shown that β chain cDNA cross-hybridises with δ chain mRNA, suggesting close homology between the mRNAs coding for δ and β chains. If the β chain genes were deleted in homozygous β^0 -thalassaemia, DNA from such a patient would have half the normal number of ' β -like' DNA sequences ($\beta + \delta$ genes) since each gene appears (from family studies) to be present as only a single copy. This difference, as shown by Kan *et al.* for their DNA mixtures, comes close to the limits of reproducibility. Homozygous ($\delta\beta$)⁰-thalassaemia has been described but this condition is exceedingly rare

and DNA from these patients has not yet been available for analysis. Ottolenghi *et al.* (Proc. natn. Acad. Sci. U.S.A., 72, 2294; 1975) have analysed DNA from a patient doubly heterozygous for β^0 -thalassaemia and ($\delta\beta$)⁰-thalassaemia in whom none, one, two, or three of the four relevant loci could be deleted, depending on whether either or both types of thalassaemia involved gene deletion. Their results showed a definite difference between this patient's DNA and normal DNA but did not clearly distinguish among the various possibilities.

Further clarification of the genetic composition of patients with β^0 -thalassaemia will probably occur only when DNA from individuals with homozygous ($\delta\beta$)⁰-thalassaemia can be obtained, or when the content of human globin sequences can be amplified by integration of human globin DNA into bacterial plasmids. The recent progress achieved with α^0 -thalassaemia, however, is encouraging. The molecular basis of these disorders appears to be understandable in terms of the number of α chain loci deleted from the genome. Perhaps more importantly, the investigation of these syndromes has shown that the same techniques can be applied to resolve issues relating to the number, content, and organisation of globin genes in the normal individual.

actions in free space, on the other hand, must conserve energy and momentum exactly so that they are on the energy shell. A phenomenological nucleon-nucleon potential fitted to the two-nucleon data does not necessarily have the correct off-energy-shell behaviour, and so may not be correct for calculating interactions inside the nucleus when other nucleons are present.

Third, the purely mathematical difficulties involved in calculating the properties of a many-body system from a knowledge of the two-body interaction are so severe that only approximate results can be obtained for any but the simplest system or the simplest nuclear property. It is for this reason that the most important information is obtained from studies of the two bound three-nucleon systems, the helion and the triton. Here it is possible to obtain quite accurate results using a variational calculation or by the methods of Faddeev.

Finally, there is a difficulty in principle that would still remain even if we had a complete knowledge of the nucleon-nucleon interaction and could overcome the difficulties of calculating from it the properties of many-body systems. This is that there may be forces that only come into play in the presence of three or more nucleons. By definition we can never find out anything about these forces by studying the interactions of two nucleons. Indeed, assuming that we can overcome the other difficulties, we can obtain some indication of the importance of three-body forces by seeing how well we can calculate the properties of a three-nucleon system using only two-body forces. If there is a discrepancy, indicating the presence of three-body forces, a three-body force can be included in the interaction and the calculation repeated to see if this gives agreement with the data.

Such three-body forces can be chosen phenomenologically, but here the difficulty is that there are many possible forms, for example $V_3f(r_{12}+r_{13}+r_{23})$ or $V_3f(r_{12}r_{13}r_{12})$, where r_{12} is the distance between particles 1 and 2 and so on, and it is not clear what is the best form to take. An alternative approach is to try to calculate the three-body force from meson theory, and use this in calculations of nuclear structure.

In the last few years there have been several sophisticated calculations of the properties of the triton using the best phenomenological potentials fitted to the two-nucleon data, and it has been found that they all give a binding energy that is lower than the experimental value by one or two MeV. Additional calculations show that the binding energy can be varied by as much as 3 MeV by altering the

Nuclear three-body forces

from P. E. Hodgson

ONE of the main aims of nuclear physics is to understand the structure of nuclei in terms of the interactions between the constituent nucleons. The nucleon-nucleon interaction may be studied by measuring the cross sections, polarisations and other characteristics of the scattering of free nucleons, in particular proton-proton and proton-neutron scattering. (It is not practicable to measure neutron-neutron scattering directly since suitable targets cannot be made.) Additional information is obtainable from studies of the only bound two-nucleon system, the deuteron. Very many studies of the nucleon-nucleon interaction have been made over the years in these ways, and the results can be represented by a phenomenological potential that depends on the distance between the two nucleons and on their spins. These potentials cannot be deduced from the experimental results; instead a trial form of the potential is postulated using forms suggested by meson theory and its parameters are adjusted to give the best fit to the data. The potential is thus not unique, and in fact several potentials have been found that give a good overall fit to the data on the

interaction of two nucleons.

The next stage is to use this potential to calculate the properties of nuclei consisting of three or four nucleons, and to see how well the results agree with measurements on the nuclei themselves. There are several difficulties in carrying out this programme. First our knowledge of the nucleon-nucleon interaction is incomplete and subject to ambiguities. This is not too serious since it is possible to compare the results obtained by using different nucleon-nucleon interactions and if they are essentially the same it is reasonable to assume that the differences between the interactions are not important for the nuclear property under consideration.

Second, in a many-body system a nucleon-nucleon interaction takes place in the field of other nucleons, which can absorb momentum. A collision can then occur in which energy is not conserved by an amount ΔE , provided it takes place in a time Δt related to the energy uncertainty by $\Delta E \Delta t < \hbar$. This is expressed by saying that nucleon-nucleon interactions inside the nucleus can take place 'off the energy shell'. Nucleon-nucleon inter-

off-energy-shell behaviour, but if this is done so as to give the correct binding energy the charge distribution no longer agrees with that found from electron elastic scattering. On the other hand if the off-energy-shell behaviour is adjusted to give correct charge distribution the binding energy becomes unacceptably low. It is thus not possible to obtain a simultaneous fit to the binding energy and the charge distribution by varying the off-energy-shell behaviour.

These calculations therefore suggest that possibly the three-body forces contribute significantly to the structure of the triton, and recently Yang (*Phys. Rev.*, **C10**, 2067; 1974) has made a detailed calculation from meson theory to investigate this. He took into account the emission and absorption of pions to lowest order as well as exchange of a ρ meson with a pion and the scattering of a nucleon into an N^* excited state at 1,236 MeV. He then evaluated the contribution of the resulting three-body potential to the binding energy of the triton using a variational triton wave function and found that it gives an important contribution of 2.32 MeV to the bonding energy. This is indeed more than enough to bind the triton, the excess being about 0.3 to 0.8 MeV, but it is likely that this is due to higher-order processes not taken into account by Yang.

These calculations suggest that the major part of the discrepancy in the calculated binding energy of the triton can be accounted for by taking three-body forces into account. Furthermore, the contribution of the three-body forces is appreciable, amounting to about a third of the total binding energy. This conclusion is highly unwelcome, since it suggests that the three-body forces, which are complicated and difficult to handle, are likely to be important in a wide range of nuclear structure calculations.

The current work on proton knock-out reactions can also give information on the importance of three-body forces. Koltun and others have shown that if only two-body forces operate the total energy of the nucleus may be expressed as a sum of the nucleon removal and kinetic energies over all the occupied orbits. A recent analysis of experimental data for the $(e,e'p)$ reaction on ^{12}C at 497 MeV (*Nature*, **250**, 105; 1975) showed that for protons this sum rule gives -4.0 ± 0.5 MeV for the mean binding energy per proton compared with the accurate value of -6.93 MeV obtained from the nuclear masses with Coulomb corrections. One possible explanation is that there are some fragments of the single-particle strength so far away from the main peak that they escape detection, so that the real

centroid energy is appreciably different from the apparent centroid energy. Another explanation is that three-body forces, which were not taken into account in the derivation of the sum rule, are indeed important. A rough estimate however indicated that the contribution of the three-body forces is small, so it was concluded that the former explanation is the correct one. The results of Yang now make it likely that three-body forces must be taken into account in the derivation of the sum rule.

Another indication of the importance of three-body forces comes from the calculations of Blatt and McKellar (*Phys. Lett.*, **52B**, 10; 1974). They studied the contribution of three-body forces to the binding energy of nuclear matter, and found that it is enhanced if the nucleon-nucleon correlations are taken into account. In this calculation

they used the correlation function derived from the Reid soft core potential, that has been obtained by systematically fitting a large body of data on nucleon-nucleon interactions. Their most surprising result is that the two-pion exchange three-body forces contribute as much as 6 MeV to the binding energy of nuclear matter. This result shows that the Reid potential overbinds nuclear matter when three-body forces are included. Since higher order three-body force terms and four-body force terms are likely to be negligible this indicates that the Reid potential needs to be modified to bring it into accord with all the data.

All these calculations indicate that it is not justified to neglect the contributions of three-body forces, and that they will now have to be taken into account in many nuclear reaction and nuclear structure calculations.

Deterministic models with chaotic dynamics

from Robert M. May

In nature there are many situations where population growth is a seasonal thing, with no overlap between generations. For instance, many temperate zone insects produce one short-lived adult generation each year, and the population trajectory consists of a sequence of discrete points. Periodical cicadas, with 7, 13 or 17 year intervals between generations, are an extreme example. In mathematical models for such systems, time is a discrete rather than a continuous variable, and one has difference equations rather than differential equations: the population at generation $t+1$, N_{t+1} , is related to that at t , N_t , by some equation of the form

$$N_{t+1} = F(N_t) \quad (1)$$

Li and Yorke (*Am. Math. Monthly*, in the press) have recently proved a remarkable theorem about any such difference equation. If there is a three-point cycle (that is, a solution such that $N_{t+3} = N_t \neq N_{t+1} \neq N_{t+2}$), it necessarily follows that for the same parameter values there are cycles of every integer period, and furthermore there exist an uncountable number of initial points for which the system is not even asymptotically periodic! Li and Yorke aptly entitle their paper "Period Three Implies Chaos".

Specifically, consider the equation

$$N_{t+1} = \lambda N_t \exp(-a N_t) \quad (2)$$

This describes a population which at low densities grows by the multiplicative

factor λ ($\lambda > 1$), but at high densities has a propensity to population decrease (the exponential form being plausible if epidemics tend to sweep the population at high densities). This equation has arisen as a model for fish (Ricker, *J. Fish. Res. Bd. Can.*, **11**, 559–623; 1954), periodical cicadas (Lloyd *et al.*, unpublished), and insect populations in general (Moran, *Biometrics*, **6**, 250–258; 1950; Cook, *Nature*, **207**, 316; 1965). The full complexity of its dynamical behaviour has, however, only recently been laid bare (May, *Science*, **186**, 645–647; 1974). For $7.34 > \lambda > 0$, the population is stable about a constant equilibrium value, to which it will tend to return if perturbed. Beyond $\lambda = 7.34$, this stable point bifurcates, and the population alternates up and down in a stable two-point cycle; this two-point cycle in turn becomes unstable, giving way to a stable four-point cycle, then an eight-point cycle, and so on; eventually the range $14.77 > \lambda > 7.34$ is filled by a hierarchy of stable cycles of periods 2^n . For $\lambda > \lambda_c = 14.77$ a chaotic regime is entered: for any given value of λ there is an infinite number of different periodic trajectories, along with an uncountable number of initial points for which the system never settles into any cycle. Further into this chaotic region, for $\lambda > 22.25$, there is a three-point cycle and consequently every integer period is represented.

At this point, proud owners of sophisticated pocket calculators may care to pause and verify some of these statements.

The above phenomena are not patho-

logical consequences of the particular equation (2), but are generic properties of any difference equation (1) describing a population with a propensity to grow at low densities, and to crash at high densities. Among many other examples which could be culled from the entomological literature is

$$N_{t+1} = a N_t - b N_t^2 \quad (3)$$

This could be regarded as the simplest nonlinear difference equation (see, for example, Chaundy and Phillips, *Q. J. Math.*, 7, 74–80; 1936). It has a spectrum of dynamical behaviour similar to equation (2), namely a stable point if $3 > a > 1$, stable cycles of period 2ⁿ if $3.57 > a > 3$, chaos for $4 > a > 3.57$ (and here N tends to $-\infty$ for $a > 4$).

Practical questions aside, these equations have intrinsic mathematical interest in providing simple examples of multiple bifurcation processes. Other examples are much more recondite and intractable.

Another way of describing the chaotic regime is to observe that arbitrarily close initial population values can lead to population trajectories which, as time goes on, diverge widely. Even if we have a model which is very simple and fully deterministic, with all parameters assumed to be constant and exactly known, the future is unpredictable. As emphasised by Oster, the dynamics of the system are in many respects indistinguishable from the sample function of a random process, and are best described in stochastic terms.

At present, apparently random fluctuations in animal populations are attributed to environmental vagaries, or to sampling errors. It may be that in the real world the nonlinearities in population growth equations are not sufficiently severe to carry them into the chaotic regime, but the possibility at least deserves attention. Other areas in which similar difference equations have recently arisen are: epidemiology (Hoppensteadt, *CBMS Regional Conf. Ser. appl. Math.*, 20, 1975); demography (for example, Lee, *Demography*, 11, 563–585; 1974); macroeconomics (for example, in non-linear generalisations of the Harrod-Domar model); genetics (Rocklin and Oster, *Am. Nat.*, in the press). In all these examples, the possible occurrence of chaotic solutions needs to be considered.

Chaotic dynamics arising from bifurcations in deterministic models have been noted in other situations. Thus Lorenz (*Tellus*, 16, 1–11; 1964) has discussed a set of three coupled nonlinear differential equations as a very much oversimplified model of convection processes in the atmosphere, and has drawn from the ensuing chaotic solutions the moral that long-term weather forecasting may be infeasible even if one had a simple deterministic model. The details of the bifurcation process here are much more complicated than in simple equations such

as those above, and are still being unravelled (for example, McLaughlin and Martin, *Phys. Rev. Lett.*, 33, 1189–1192; 1974). Likewise Ruelle and Takens (*Comm. Math. Phys.*, 20, 167–192; 1971) have proposed a set of four deterministic coupled nonlinear differential equations, with a complex bifurcation structure, as a paradigm for the transition from laminar to turbulent fluid flow. And in an ecological context, Hassell and Comins (*Theor. Pop. Biol.*, in the press) have followed up the above work by exploring the way chaotic population dynamics arise somewhat more easily in discrete time models of two competitors, and Beddington, Free and Lawton (*Nature*, 255, 58–60; 1975) have done a similar job for discrete time predator-prey models.

Apart from implications in specific scientific contexts, there are general morals to be drawn from the rich spectrum of dynamical behaviour of simple equations such as (2) and (3). Most students come away from elementary mathematics and physics courses (which emphasise linear models) with the impression that simple deterministic models have simple deterministic dynamics. This is, in my view, a very misguided prejudice to bring to biology, economics or sociology, where the simplest models are likely to be non-linear, and consequently can have strange dynamics. Equation (3) could be studied by high school students before they met differential calculus: it would do them good.

I conclude with some cocktail party chit-chat. Note that the properties of equations such as (2) and (3) in the chaotic regime help to reconcile the Calvinist view of a foreordained, deterministic Universe with the appearance of free will in the world: the Calvinist God knows the exact initial conditions. Less flippantly, one may recall the objections to the probabilistic foundations of modern quantum mechanics, summarised by Einstein's objection: "God does not play dice". Many people have speculated that there may be 'hidden variables', and an underlying deterministic structure. Equations such as (2) and (3) can provide very simple examples of fully deterministic systems whose dynamics are best described probabilistically, and may go some little way toward adding substance to these speculations.

New confidence in ancient pollen data

from Peter D. Moore

THE pious hope of all who study past vegetation by the use of pollen analysis is that the abundance of the pollen of a particular taxon within a deposit bears a close relationship to the contemporary abundance of that taxon in the



A hundred years ago

In connection with the Arctic papers of the Geographical Society, we recently referred to speculations on the condition of the interior of Greenland. The August number of the *Mitteilungen* will contain a paper by Dr Rink on this subject, and on the possibility of crossing Greenland. The following are his principal conclusions:— 1. The so-called interior ice is probably only a wall or rind, inside which may be found valleys free from snow and ice, and possibly even wooded. 2. All Greenland, probably, consists of a number of islands soldered together by the universal ice covering. 3. Most probably in two or three places, where the ice-fjords still dis-embogue, in earlier times a sound must have extended right across from the west to the east coast. 4. Glaciers and permanent snow are probably on the increase all over the land. 5. Floating icebergs are detached from the land by a sort of fall or downflow of the land-ice glaciers. Dr Rink thinks that by means of properly constructed sledges drawn by men, and by carefully selecting a route and establishing suitable stations, the Greenland continent might be crossed from coast to coast.
from *Nature*, 12, 241; July 22, 1875.

vicinity of the deposit. Yet, despite the palaeoecologist's declared belief that the present is the key to the past, the study of the relationship between modern pollen rain and the vegetation responsible for it has been sadly neglected. As a result, most of the vegetational interpretations appended to accounts of pollen stratigraphy are largely based upon glib and unjustified assumption.

But information on current pollen rain is now being collected, and many palynologists must have breathed sighs of relief when they saw data such as that published by Lichti-Federovich and Ritchie (*Rev. Palaeobot. Palynol.*, 7, 297; 1968). These showed that surface sediment samples taken from the major vegetational zones of northern North America contained pollen which broadly corresponded in composition to the vegetation of those zones. Treeless tundra sites proved, predictably, an exception since long-distance transport, particularly of gymnosperm pollen, was a major component of the pollen rain.

Since that study, information on pollen rain has been collected elsewhere from a variety of vegetation types. Webb, for example (*Ecology*, 55, 17:

1974) sampled the surface sediments of 64 lakes, evenly distributed over a study area in lower Michigan. He could draw fairly precise isofrequency contours of the major tree pollen types across lower Michigan and these corresponded reasonably well with relative abundance maps of the trees concerned. The exceptions were pine and poplar, which were respectively over- and under-represented in the surface sediments. The general north-south gradient in vegetation and in pollen spectra was confirmed by principal components analysis.

Information of this sort causes British palynologists to be filled with a new confidence in the usefulness of that painstakingly accumulated assemblage of fossil pollen data (now safely stored in the Quaternary data bank at Cambridge). As a result some of these data have recently been dusted down and subjected to fresh scrutiny and analysis by Birks, Deacon and Peglar (*Proc. R. Soc. Lond.*, **B189**, 87; 1975). Basically they have attempted to reconstruct pollen rain maps of the British Isles of 5,000 years ago, using isofrequency contours based on the relative proportions of tree pollen in contemporary peats and lake sediments.

The choice of the period for study, around 3,000 b.c., is a consequence of the recent collation by Smith and Pilcher (*New Phytol.* **72**, 903; 1973) of radio-carbon dates from the major pollen stratigraphic horizons in Britain, which have been used as the basis for the Godwin zonation system. As expected, their collation served to demonstrate the metachroneity of these boundaries in different parts of the British Isles, with the notable exception of the elm decline horizon. This horizon (formerly used to demarcate Godwin's zone VIIa from zone VIIb—the 'Atlantic-Sub-Boreal transition') was generally dated at between 5,400 and 5,100 b.p. throughout most of the British Isles. It therefore provides a very useful datum horizon in pollen stratigraphic profiles, which is why Birks *et al.* have chosen the levels preceding the elm decline for their analysis.

One feature to emerge from their maps is the paucity of sites from which pollen data are available in the midlands and the south of England compared with the north, the west, and Ireland. This lack of sites means that the placing of isopollen contours in the midlands and south has to be somewhat arbitrary.

The overall picture conveyed by the maps is one of a vegetational gradient within the British Isles in which birch and pine are concentrated in northern and eastern Scotland (with an interesting 'island' of pine in East Anglia),

oak in northern England, elm and hazel in Ireland and the south-west, and lime in south-east Britain. This north-west-south-east gradient is confirmed by principal components analysis of the data; 54% of the total variance in the data is accounted for by the first three principal components, which reflect the major vegetational regions of the British Isles when plotted on a map. The remaining variance is presumably due to local site factors which produce heterogeneity within the regional gradient.

Although there is a greater abundance of data since Godwin (*New Phytol.*, **39**, 370; 1940) first performed this type of mapping exercise, the conclusions are very similar. What has changed is the confidence with which one can interpret these pollen maps of the period prior to the advent of Neolithic man.

Eukaryote transcription initiation sites

from Harry R. Matthews

THE control of gene expression in higher organisms remains a mystery in molecular terms. One of the stages at which control can be exercised is transcription of the gene (DNA sequence) into an RNA sequence which can then be processed and eventually translated. The cell controls transcription in two ways: it avoids transcribing DNA which has a function not expressed through transcription; and it concentrates transcription on those genes that are active or that are controlled at a later stage in expression.

DNA is transcribed by an enzyme, DNA-dependent RNA polymerase. Higher organisms possess several such enzymes which are usually classified, operationally, on the basis of resistance to the drug, α -amanitin. Class A, or I, enzymes are resistant; class B, or II, enzymes are very sensitive; and class C enzymes are fairly sensitive. Class A enzymes transcribe the genes for ribosomal RNA and are located in the nucleolus; class B enzymes probably transcribe the bulk of the remaining expressed DNA; and class C enzymes may transcribe the reiterated genes for tRNA and 5S RNA. How does RNA polymerase B know which DNA sequences to transcribe? It seems unlikely that the enzyme itself is responsible for the entire range of tissue-specific transcription that can be observed by molecular hybridisation techniques since, for example, some tissue-specific transcription can be observed *in vitro* using chromatin from different mammalian tissues and bacterial RNA

polymerase. However, especially in the light of reports that a large proportion of DNA in chromatin is available for protein interactions, RNA polymerase B could well need a built-in specificity for particular initiation sites on DNA in order to limit the amount of DNA that needs to be covered by the tissue-specific control elements.

Evidence for such specificity is accumulating. Meilhack and Chambon (*Eur. J. Biochem.*, **35**, 454; 1973) and, now, Cedar (*J. molec. Biol.*, **95**, 257; 1975) have shown that there is a limited number of initiation sites for RNA polymerase B on homologous calf thymus template. Meilhack and Chambon allowed initiation to occur by incubating DNA and polymerase in the absence of nucleoside triphosphates, then adding the rifamycin derivative AF/013 which prevented further initiation and nucleoside triphosphates which permitted chain elongation. The number of RNA chains synthesised was determined from the incorporation of γ - 32 P-purine triphosphates. They found, on average, one initiation site per 60,000 nucleotide pairs. Cedar has confirmed this result by using high salt conditions to prevent re-initiation of RNA chains after a pre-incubation in low salt, a method first used by Hyman and Davidson in a prokaryote system. He found one initiation per 30,000 nucleotide pairs. Cedar also counted his RNA chains by measuring 3 H-UTP incorporation and dividing by the RNA molecular weight obtained by sucrose gradient ultracentrifugation. This method gave one initiation per 40,000 nucleotide pairs.

The figures are acceptably close and correspond to 150,000 polymerase B initiation sites per diploid calf nucleus. The upper limit, given by population genetics, for the number of independent genes per haploid genome is 50,000, that is, 100,000 per diploid nucleus. The number of sites is intriguingly close to the number of genes but, wisely, nobody is jumping to conclusions at this stage. In a long nucleotide sequence a given sequence of seven nucleotide pairs has, to a first approximation, a probability 1 in 65,000 of occurring by chance. Cedar found one initiation site per 50,000 nucleotide pairs with calf thymus B and *E. coli* DNA so the polymerase may recognise a nucleotide sequence about seven nucleotides long that occurs by chance in *E. coli*.

The number of initiation sites is further restricted in chromatin. On average, one initiation was found for every 400,000 nucleotide pairs in calf thymus chromatin in solution, which implies 15,000 initiation sites per diploid nucleus in calf thymus. This is comparable to the estimated number of active genes although it may be a little higher. The

number of initiation sites could be higher than the number of genes expressed if the cell transcribes sequences that do not contain a gene or if many genes have multiple initiation sites. It is also possible that some or all of the initiations observed *in vitro* do not occur *in vivo*. In order to investigate the relationship between the *in vivo* and *in vitro* RNA transcripts their nucleotide sequences must be studied. It is becoming possible to do this directly and some 5'-terminal sequences of nuclear RNAs are appearing (see *Nature*, **255**, 9; 1975). Alternatively molecular hybridisation techniques can be used, especially when a particular gene can be isolated or a cDNA copy of a pure messenger RNA obtained, which can then be used as a highly specific probe for the transcription of a particular gene.

Breeding for protein yield and quality

from Donald Boulter

THE relative importance of protein, as opposed to protein/calorie deficiency, on a world scale, continues to be debated (P. Payne, *New Scientist*, 7 November, 1974), although most of the arguments are of the paper and pencil calculation type. Whatever the final outcome of this debate, it should not obscure the fact that most field workers agree that there is a need to increase the productivity and to stabilise the yield of high protein crops, particularly of legumes since these can fix atmospheric nitrogen.

In diets in many developing countries, legumes play a significant part in supplying protein, as well as in improving soil fertility, and the first priority is to stabilise yield by breeding more resistant lines and then to increase productivity by improved management and restructuring of the plant type. A likely result of such improved legume varieties is an increase in protein yield. Where small amounts of food are required, for example by young children, the total amount of protein consumed is small and any nutritional deficiency of that protein prevents complete utilisation of it. In this situation an improvement in protein quality is essential; this applies both to cereal- and legume-based diets. With adults the available information on diets is insufficient to show the extent of the need for improved protein quality. Also, alternatives such as influencing dietary patterns so that protein from one source may nutritionally supplement that from another, will also have a role. However, in diets in which the main staple is cassava and possibly in those using other roots or

tubers, the quality of the protein consumed may be inadequate for its complete utilisation; here sulpho-amino acids are likely to be first-limiting.

Many of the problems involved have been discussed recently at the Third Research Coordination Meeting of the FAO/IAEA/GSF Seed Protein Improvement Programme, held in Hahnentlee, FDR, 5-9 May 1975.

The plant breeders at the meeting were firm in their belief that, to be acceptable to farmers, varieties improved with respect to protein quantity and quality must have high and dependable yields. The one exception to this generalisation is where such varieties might be grown as premium crops where quality brings additional income, a relatively uncommon situation particularly in developing countries. The long-assumed negative relationship between protein yield and grain yield is now known not to be generally valid and in wheat and rice, for example, improvement in both characters has been achieved simultaneously. As long as the demand in a cereal crop for protein content is not excessive, a few percentage points increase, significant advances can be made without sacrificing grain yield. In soyabean, restructuring has achieved very high protein content and high yields.

The same cannot be said, at this time, for protein quality. The experience with high lysine maize, barley and sorghum all point to somewhat reduced yields of grain when genes for increase in lysine are included in a genotype. But it was generally agreed that attempts to incorporate high lysine genes into other genetic backgrounds were only beginning.

J. Axtell (Purdue University) described investigations on natural and induced genes for high lysine in grain sorghum. In this work, a population of seeds was treated with the mutagen diethyl sulphate and selections were made in later generations for the opaque endosperm character by passing seeds over a light box. Only certain opaque endosperm genotypes also carried the high lysine character. The desirable endosperm types have been grown for additional generations, but some showed highly deleterious characters as well. Nevertheless, a high lysine endosperm type with good growth characteristics has been recovered (P-721).

R. A. Olson (University of Nebraska) had documented considerable variability in soil conditions in fields used for growing breeding materials for protein improvement, and showed that, probably as a result, the protein content of the plants varied. In the same vein, W. Gottschalk (University of Bonn) presented results from field pea

genotypes in which ranking in both protein content and grain yield were shown to fluctuate widely from year to year. This result is in contrast to those obtained with wheat and barley trials carried on elsewhere, wherein the rankings remained relatively constant between locations and years. Walther (Grünbach, FDR) presented results with barley showing that in an experiment with adequate replication in seasons and locations, it is possible to elucidate plant responses with respect to yield and protein content with apparent reliability. All of these results taken together re-emphasised the difficulties in protein breeding due to gene/environment interactions, and the necessity for interpreting early results cautiously.

L. Munck (Carlsberg Laboratories, Copenhagen) considered the prospects for the use of high-lysine grains in the animal feeding industry. First, the industry must appreciate the nutritive advantage of high-lysine grains when considering the inventory of the available feeding materials. Second, evaluating procedures need to be available; for example, Munck suggested dye-binding, used in parallel with Kjeldahl nitrogen determination, as a possible means of recognising high-lysine feedstuffs. Only then would feedback pressure on the plant breeders lead to the general inclusion of the high-lysine character in their breeding objectives.

It is well known that legumes are nutritionally deficient in sulpho-amino acids, and D. Boulter (University of Durham) pointed out that it is necessary to consider both sulpho-amino acids, methionine and cysteine, in work on breeding for improved protein nutritive value in legumes. It seems possible to use total seed sulphur as a coarse ranking indicator of the sulpho-amino acids for screening of some legumes. In those legumes which contain relatively large amounts of S-methyl-cysteine, sulphur must be determined after removal of this amino acid by extraction with 70% alcohol. Both Boulter and R. M. Gillespie (CSIRO, Glen Osmond, South Australia) suggested that there are reasonable prospects for being able to breed for improved nutritional value by altering the proportions of the major seed proteins which contain different amounts of sulpho-amino acids. In Boulter's view however, present indications are that the range of variation in the protein sulpho-amino acid content of legumes in breeding programmes is small enough to warrant considerable effort in the assembling of world collections.

A new dimension to this problem was furnished by the work reported by Gillespie (see Blagrove and Gillespie, *Aust. J. Plant Physiol.*, **2**, 13-27; 1975;

Gillespie and Blagrove, *Aust. J. Plant Physiol.*, **2**, 29–39; 1975). They have found that in lupins the relative proportions of the major seed proteins can be changed up to a point by supplying sulphur at different levels; in high-sulphur treatments the proportion of a high-sulphur protein was increased. Thus, there is a strong gene–environmental interaction, although whether the effect is directly on gene expression at the transcription or translation levels, or whether it operates by a changed physiology, is not clear. It will be of great interest to see how general these results are for other legumes.

T. Behre (Debre Zeit, Ethiopia) reported a breakthrough on breeding *Eragrostis tef*, which represents a significant source of protein in Ethiopia. Up to now, all attempts at controlling the pollination of this crop have been unsuccessful. But as a result of studying the basic reproductive process of the plant, it has been possible to perform hand pollinations, so that normal procedures of plant breeding may be applied and it should now be possible to make improvements in yield through breeding.

In the realm of improved techniques for screening and evaluating the nutritional quality of advanced lines, there was an encouraging report concerning the use of a fluorescence technique for the specific determination of lysine in ground samples of grain. Less advanced is some work on a similar technique for tryptophan determination. Both of these developments were reported by A. K. Kaul (Institut für Strahlenbotanik, Hannover).

A number of contractors from developing countries reported progress on the development of improved protein genotypes in several crops, including bread and durum wheat, rice, barley and several legumes. Because of the early stage of the work, no attempt is made to detail the results here, but a general feeling of optimism prevailed. For example, H. K. Jain (New Delhi) was convinced that tropical legumes as a group were excellent materials in which great improvements could be achieved.

A homogeneous Universe?

from Malcolm MacCallum

THE simplest cosmological models assume the Universe to be spatially homogeneous and isotropic. Isotropy, the equivalence of all directions from us, is on a fairly firm experimental basis provided by observations of the cosmic microwave background, the X-ray background and so on, although there are contradictory results (Brown, *Mon.*

Not. R. astr. Soc., **138**, 527; 1968; Wilson, *Mon. Not. R. astr. Soc.* **155**, 275; 1972) demanding further investigation. However, isotropy does not directly imply homogeneity, the equivalence of all points in space, for we might simply be at 'the centre of the Universe'. There is a strong anti-geocentric prejudice which favours homogeneity, but a lack of observational evidence.

The prejudice itself is recent. Only in 1918 did Shapley find the Sun was not at the centre of our Galaxy, three and a half centuries after Copernicus's discoveries. It was 1924 when Hubble proved some 'nebulae' were galaxies and 1952 when Baade's revision of the cosmic distance scale showed other galaxies could be as large as our own. The 'Copernican principle' and its strong form, the assumption of homogeneity, have subsequently been almost articles of faith, especially as they have the practical advantage of simplicity.

This attitude is reinforced by the difficulties of observationally checking homogeneity, arising because it involves a knowledge of conditions at all points, however distant. All one can hope to say is that any inhomogeneity has a scale greater than our range of observation. If the latter included our whole past we would know all we really require. But the finite velocity of light means we must compare distant objects as they were in the past and not as they are now, so we need to know about the evolution of both individual astrophysical objects and the Universe as a whole to make the comparison.

In spite of these difficulties, our view about homogeneity is naturally influenced by knowledge of the cosmic structure in our vicinity. The most obvious feature to test is the distribution of galaxies. It is now well known that galaxies are clustered and so not homogeneously distributed. de Vaucouleurs (for example in *Science*, **167**, 1203; 1970) has expounded a theory of a hierarchy in which clusters are grouped in superclusters and so on. Peebles and collaborators (*Astrophys. J.*, **196**, 1; 1975) found no evidence of a hierarchy in their extensive statistical investigations of catalogues of galaxies. They concluded that there is a continuous spectrum of clusters on scales $10/h$ kpc to $5/h$ Mpc, where h represents the time cosmic distance scale in suitable units and 1 pc is roughly 3×10^{13} km. Current estimates of h are roughly in the range $\frac{1}{2}$ to 1. This distribution could be the effect of gravitational attraction within initial accidental density enhancements, the clusters themselves being homogeneously distributed.

Turner and Gott (*Astrophys. J.*, **197**, L89; 1975) have just announced a new development. They were studying the problem of the non-luminous 'missing mass' that may be required to gravi-

tationally bind clusters of galaxies, by examining the dynamics of small groups of galaxies. To find such groups they took all Northern Hemisphere galaxies brighter than fourteen magnitude (which corresponds to a distance of order roughly 100 Mpc) at more than forty degrees from the galactic plane, and separated these into two classes according to whether their nearest neighbour was at an angular separation greater or less than 45 minutes of arc. They noted that the more isolated class appeared to be homogeneously distributed in the sky, and thus tried applying Peebles's statistical methods to each class separately. They found the 'isolated' class, about 40% of the total, were indeed homogeneous, once effects of galactic obscuration and statistical noise had been allowed for. The other class however showed strong clustering on scales up to eight degrees.

This result, which invites further examination, will, if confirmed, leave us awkwardly poised. Each of the two classes separately is reasonably explicable, but Turner and Gott show from velocity and brightness data that they must be intermingled, and how one class became clumped while the other did not is a mystery. Once again nature proves to be more complex than was thought.

Of bedsteads and neutrons

from James Binney and Philip Candelas

INTEREST in the interaction of quantum theory and gravitation has been growing recently. Following Hawking's sensational prediction (*Nature*, **248**, 30; 1974) that black holes may emit photons and other elementary particles, several recent publications have discussed the importance of quantum effects in strong gravitational fields (see review by DeWitt, *Physics Reports*, in the press; and *Quantum Gravity: An Oxford Symposium*, edit. by Isham, Penrose, and Sciama; Oxford University, 1975).

Just to make sure we have the basics right Colella, Overhauser and Werner (*Phys. Rev. Lett.*, **34**, 1472; 1975) have been checking up experimentally that the de Broglie waves associated with neutrons really are refracted by a gravitational field in the same way that proton wavepackets are refracted by an electric field. Colella and co-workers have cut from a single crystal of silicon a device which operates much as a Young's double slit experiment. Bragg diffraction in one part of the crystal splits a neutron beam into two, each being subsequently diffracted back towards the other by another part of the crystal. Colella *et al.* then monitor

the intensity of the signal produced by the interference of these two beams in a third part of the crystal. They find that the relative phase of the two interfering beams changes as elementary quantum mechanics predicts it should when the apparatus is rotated so as to alter the relative inclination of the two beams to the horizontal.

Of course one is not very surprised that the result of a low-energy experiment of this type is accurately predicted by conventional quantum theory. It is unfortunate that weak gravitational fields should be the only ones accessible to experiment since recent remarkable results due to Hawking and Beckenstein indicate profound interrelations between quantum theory, gravitation and thermodynamics.

Hawking discovered that, in the case of a realistic black hole formed by stellar collapse, effects to do with the quantum fluctuations of the vacuum cause real particles to be produced and emitted to infinity as a constant flux. What is really astonishing about Hawking's result is its form. The outcome of his highly sophisticated analysis is that black holes should radiate photons and other particles precisely as if they were black bodies of definite temperature. The temperature is inversely proportional to the mass of the hole.

Surprisingly, this result is essentially that suggested earlier by Beckenstein (*Phys. Rev.*, **D7**, 2333; 1973) from an entirely different, very classical argument: Take a large, brassy Victorian bedstead and consider it carefully for a moment. You observe four legs, many knobs and springs. Indeed if you are a connoisseur of such things you might well guess that it was manufactured in Bristol in 1864. Toss this same bedstead into a black hole and all this information is suddenly lost to you. The only differences that you observe in the hole are small changes in its mass and angular momentum. But of course a loss of information implies an increase in entropy, an effect which one can quantify. In this way Beckenstein was able to put a value on the entropy of a black hole, which turned out to be proportional to its surface area. From this followed in a formal way a value for its thermodynamic temperature. What Hawking showed us was that this formal temperature is perfectly real.

These two results fit beautifully with work by Bardeen, Carter and Hawking (see for example Carter's contribution to *Black Holes*, edit. by DeWitt and DeWitt; Gordon and Breach, 1973) who showed that changes in the configuration of a black hole are constrained by a series of laws analogous to those of thermodynamics. This similarity was at first thought entirely

formal with the inverse of the black hole mass having the role of temperature and the area of the black hole behaving like entropy. Now in the light of the work by Hawking and Beckenstein we see that there is a complete identification of black hole parameters and thermodynamic variables.

It is very encouraging that a field as young as is the study of quantised particles in gravitational fields should already have produced a much needed piece of the jig-saw of physics. A host of cosmological problems seem to require for their solution a better understanding of these quantum processes. Perhaps with the solution of these other problems we will again obtain important and unexpected insights into the nature of things.

Spots before the eyes

from E. G. Richards

A RECENTLY published two-dimensional electropherogram shows the resolution of more than 1,100 proteins from *E. coli*. This spectacular result is to be found in a paper by O'Farrell (*J. biol. Chem.*, **250**, 4007; 1975).

Two-dimensional electrophoresis involves the separation of a mixture along the first dimension according to some molecular property of the components; this is followed by movement of the components in a perpendicular direction resulting in separation according to another property. Most attempts to apply such methods to mixtures of proteins have suffered from correlations between the two chosen properties resulting in a tendency of the components to cluster round a diagonal in the final pattern.

O'Farrell has played the simple trick of separating according to isoelectric point by isoelectric focusing in the first dimension followed by a separation according to molecular weight by SDS-polyacrylamide electrophoresis in the second. The isoelectric point and molecular weight show virtually no correlation.

To summarise his method, the separation in the first dimension is conducted in a 24% cylindrical polyacrylamide gel containing 9 M urea and ampholines to produce a pH gradient between pH 5 and 7. For the second dimension a 0.8 mm thick flat bed gel of polyacrylamide with an exponential concentration gradient and containing SDS is used. The sample to be applied is first treated with nucleases to remove RNA and DNA which otherwise produce streaking effects and then the proteins are solubilised and denatured in 9 M urea before application to the first dimension. After the first separation

the isoelectric gel is advantageously equilibrated by shaking in SDS solution before incorporation into the flat bed gel. Although this equilibration leads to some loss of proteins and some impairment of the resolution, it further reduces streaking.

The authors claim that about 70 bands can be resolved in the first isoelectric separation, and up to 100 in the second SDS dimension. Thus a maximum of 7,000 proteins could in principle be resolved though this figure may be reduced somewhat in practice for a variety of technical reasons. Nevertheless the authors have counted more than 1,100 spots obtained from the application of whole *E. coli* extracts. A rough calculation which assumes a chromosome of 4 million base pairs and a thousand of these to each protein gives an upper limit of 4,000 different proteins in *E. coli*.

Two detection systems were utilised: staining with Coomassie blue and autoradiography with the use of ¹⁴C labelled proteins. The first method can detect 0.01 µg of protein in a spot; the second, in a twenty day exposure, can detect 1 c.p.m. which could correspond to a protein which existed in but one copy per cell at realistic, albeit high, specific activities. However these detection limits are by no means the whole story since the picture is complicated when the total load of protein is raised to enable minor components to be detected. In the first place the total protein load must be kept low to avoid streaking effects and other distortions of the spot shape; in the second the size of a spot was found to increase when the amount of protein in it increased. This means that minor components located close to abundant proteins would be hidden and remain undetected.

The authors claim that replicate runs are highly reproducible provided the conditions are carefully standardised and ampholines from the same batch are used. The comparison of two electropherograms is facilitated by the characteristic patterns of spots produced.

If the author's recipes are not carefully adhered to, certain artefacts may be introduced such as streaking and multiple spots due to charge heterogeneity and solubility problems.

It would thus seem that a new tool of considerable power has arrived and it will be interesting to see what use is made of it. O'Farrell himself says that the method as it stands does not work for basic proteins such as ribosomal proteins and histones but one may guess that there are no insuperable problems in adapting it for such mixtures. Maybe the characteristic patterns of spots will also commend themselves to taxonomists.

review article

Computer-aided thought in biomedical research

W. J. Perkins & B. J. Hammond*

With the technology at present available, computers are potentially more than simply mindless calculating machines. They can now be programmed to make intelligent use of the ideas researchers have about the problems they are trying to solve.

The human mind is seldom satisfied, and is certainly never exercising its highest functions when it is doing the work of a calculating machine. What the man of science, whether he is a mathematician or physical enquirer, aims at is to acquire and develop clear ideas of the things he deals with. For this purpose he is willing to enter on long calculations and to be for a season a calculating machine, if he can only at last make his ideas clearer.

J. C. Maxwell (1870)¹.

THE relationship between the man of science and his calculating machine has undergone, particularly in recent years, a rapid evolution but it is the machine and not the man that has changed. For in spite of the almost overwhelming power of a modern computer, the scientist still simply seeks to 'develop clear ideas of the things he deals with'. The calculating power that evaded Maxwell's grasp has since become a common-place means of evaluating data from scientific experiments but it is equally well established that the computer is, at least potentially, more than an outsize calculating machine. Substance is being added to this view, albeit slowly, by research in those areas of computer science gathered somewhat uneasily under the umbrella of artificial intelligence², but for the scientific user the more exciting prospects must at present remain as unredeemed promises. It is nevertheless possible to marshal a sufficient range of techniques, both in hardware and software, to provide the researcher with an effective tool for processing ideas as effectively as he may process numerical data.

An illustration of the difference between these two activities is seen in the application of computers to the problem of virus structure. The electron microscope provides pictorial views of individual viruses seen from various angles but, because of the preparative procedures and the fact that the microscope is operating at its limit of resolution, the images obtained are difficult to interpret in terms of three-dimensional structure. The electron micrograph may, however, be scanned automatically and reduced to an array of density data in a computer, thus allowing various lengthy mathematical transforms to be applied in attempts to improve the resolution of the record, or to make symmetry relationships more explicit. It is also possible to reconstruct the three-dimensional shape of a virus from a series of electron micrographs of a given specimen taken at various tilt angles, though at present, radiation damage of the specimen limits the number of exposures that can be made and so restricts the technique to highly symmetrical structures³. This data processing approach is designed to help in building a mental picture of virus structure but it makes little use of the tentative ideas that the researcher may be carrying in his head at any time.

Ideas about the possible shapes of a virus find a more direct means of expression in the use of physical models, which act both as a focus and an embodiment of the researcher's thinking and can be changed almost at will as shortcomings of particular structures are recognised. A more sophisticated and more flexible extension of the physical model can be obtained if the model is computer-based but, particularly in the early stages of model development, there is a need for more rapid assessment of ideas than that provided by off-line computer facilities. Close interaction between modeller and model is needed if the computer is to aid and not frustrate creative thinking of the user.

Hardware and software

From the technical point of view then, it is necessary to exploit to the full those computer facilities that speed communication, in either direction, between man and machine so that a computer intended as a thinking-aid is characterised more by its range of operator input-output devices than by an impressive size. Such input devices include, in addition to the standard keyboard, simple switches and potentiometers for indicating binary conditions and analogue levels to the machine, graphic tablets for communicating line contours to the computer, and the light-pens and joy-sticks used in conjunction with output displays to signal positional information. Interactive output facilities, in addition to the standard teleprinter or its electronic equivalent, encompass various oscilloscope devices for displaying line drawings in a storage or non-storage mode and television-type displays for presenting continuous tone or coloured picture information to the user.

The provision of suitable hardware is necessary but not

Table 1 Keyboard commands for rotating computer model of adenovirus hexon

a Building instructions	
BR/X./Y./Z./	Bring a cell into position
JN/-N/φ./ψ./	Join on to cell N at given angles
SH/-N/-N1-N2/	Show number of cell N. Type coordinates N1 to N2
DE/-M/-N	Delete cells M to N
MST/M/-N	Make a structure numbered M
BRS/-N/X./Y./Z./θ./ψ./	Bring a structure into position given
BN/-N/	Add background of N dots per inch ²
RE/	Read in a structure
OU/	Output a structure
b Rotation instructions	
AX/N/φ./θ./	Set angles of axes for quadrant N
RTX/θ./	Rotate structure about X by angle θ
RTY/φ./	Rotate structure about Y by angle φ
RTZ/ψ./	Rotate structure about Z by angle ψ

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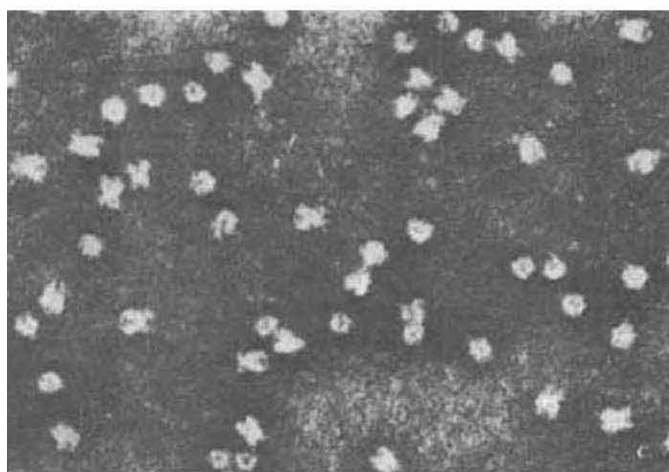
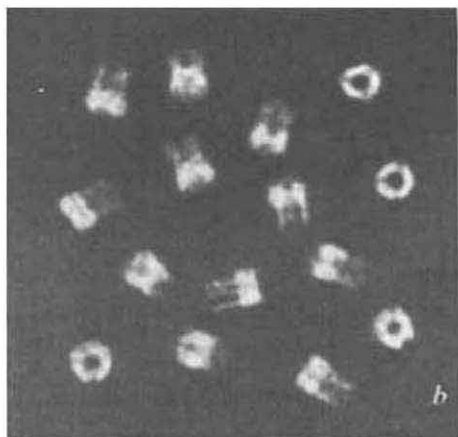
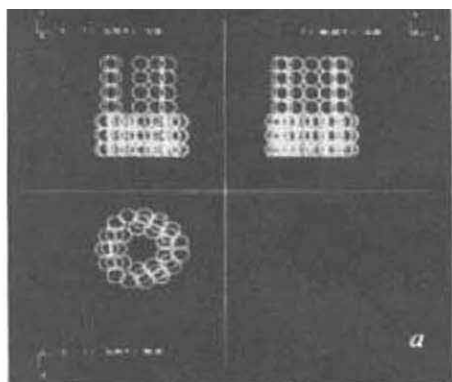


Fig. 1 *a*, Basic model of adenovirus hexon in the orthogonal planes; *b*, various orientations of the adenovirus hexon model; *c*, electron micrograph of adenovirus hexons showing diverse orientations.

sufficient to establish close interaction between user and computer; effective interaction also lies with effective programming. A difficulty arises in the fact that in the present context the models are by definition tentative, and the nature of any changes that may subsequently be required cannot be clearly foreseen, yet an interactive program should at the outset provide the user with a sufficient range of alternative model manipulations at least to launch the model on its uncharted course of development. Subsequent program changes will almost inevitably be required, either to modify the nature of the model or to provide further manipulation facilities, and the ease with which these may be implemented depends to a large extent on the degree to which the program has been

written in autonomous blocks. Modular programming provides a suitable software structure that implements the various model manipulations as a set of self-contained blocks to which changes, additions and deletions may readily be made as modelling requirements change with a model's evolution.

Interactive graphics

One basic way in which hardware and software may be coordinated to provide an aid to thought lies in the use of interactive graphics, which can best be illustrated by returning to the problem of virus structure. A modular program has been developed that allows the interactive building of tentative virus structures using spheres as basic building units. The keyboard commands for the control of model building are shown in Table 1 and the three-dimensional nature of the structure is presented to the user as orthogonal projections on a storage display screen. Starting with a blank screen the user can type bring (BR) commands to establish single spherical units at specified coordinates and further units can be attached to existing units by use of a join (JN) command. Each unit is allocated an identification number as it is entered on the screen and the identity of a specified unit can be retrieved at any time using a show (SH) command. Units can also be removed from the structure by means of a delete (DE) command. Another useful building feature is the ability to mark and store a structure using an MST command which subsequently allows the marked structure to be used again, either in its own right or as the subunit of a grander design.

These few commands, with the immediate presentation of their effects on the model, allow effective interaction between user and computer in building plausible models of virus structure; this is illustrated by Fig. 1*a* which shows a model of an adenovirus hexon, built up in this way and displayed in the orthogonal planes. The human contribution to the man-machine interaction is not only his speculative ability but his ability to compare the geometric properties of a model with the fuzzy images provided by the electron microscope. This comparison can be assisted by randomly filling the spherical building units with a suitably dense distribution of dots to indicate depth. In addition, a random distribution of dots at any selected level can be added as a background to the model. By the use of the further keyboard commands given in Table 1, the model can be rotated about all axes to present planar orientations that can be compared directly with the two-dimensional projections observed in electron micrographs of the virus specimens. The diverse patterns observed during rotation give ideas as to how other patterns might be obtained then the model can be refined to obtain a closer match. Various orientations of the adenovirus hexon model may be seen in Fig. 1*b* and related to the diverse projections observed in the electron micrograph of Fig. 1*c*. The distribution of categorised patterns in the model may also be compared with distributions in electron micrographs for different preparative procedures to assess their effect on the patterns obtained.

Three-dimensional displays

Two-dimensional projections of computer-implemented structures are progressively less useful as three-dimensional complexity increases, but there is no difficulty in transforming the data in the computer to produce a stereo display⁴. An example of this approach features molecular models of proteins where the user, viewing a large screen oscilloscope through a suitable mirror system, sees the appropriate network of straight-line bonds as a three-dimensional structure in space. The space relationships can be further clarified by rotating about orthogonal axes using manual potentiometers interfaced to the computer through A-D converters. This presentation is at first dramatic but is no more than a computer-based version of conventional ball and stick models, that is, until interactive facilities are added to the basic facility.

Consider for example the molecular conformation of complex molecules where the sequences of atomic components are largely known but where the structure still retains sufficiently numerous degrees of freedom to generate an immense number of possible second order configurations. The configurations preferred by nature are those of minimum energy but the determination of this condition for a complex molecule by an unguided search is usually rendered impracticable by the sheer number of candidates to be considered and by the numerous local minima observed⁵. In practice the researcher seeking natural conformations bounds the search according to largely empirical rules, but if a closely interactive approach is adopted, available clues from other sources may be incorporated together with experience, ideas and sometimes intuition. Three-dimensional displays of the molecular structure provide an effective focus for such interaction and control of the conformation of the structure is readily provided by manual potentiometers that specify particular bonding angles and can therefore be used to rotate one part of the structure relative to the remainder.

At any point in the interaction the user may call for energy calculations to be made corresponding with the incremented rotations of a specified bond about two orthogonal axes. This information can be presented in a variety of ways but each uses the mapping of energy values on to a plane defined by axes of the incremented variables. The more sophisticated displays present the user either with constant energy contours or with two-dimensional views of the energy surface constructed above the reference plane. In either case a rapid assessment of the current situation can be made and serves to direct the next step in pursuit of a minimum energy configuration⁶.

Conversational mode

The interactive manipulation of complex molecules places its heaviest technical demands upon communication from machine to man but in other situations, communication in the reverse direction is more important. The man-to-machine link is made through a coded instruction chosen from a prearranged list. If the idea of an instruction is, however, interpreted too rigidly, that is, if it is taken to imply an imperative requiring no response other than blind obedience, then the onus for precise expression of complex instruction formats falls entirely on the user and any operator errors will generally cause the computer program to abort. The consequent loss of rapport between man and machine can, however, be restored by upgrading the role of the computer as an instructed device, so that it apparently takes the initiative whenever a decision is required. At such times the computer explicitly invites a decision, checks the validity of the user's reply and then, by prompting, guides the user along the chosen path of action. In this way a conversational mode of control evolves in which the real prerogative of choice remains with the man, while the tedious responsibility for its appropriate expression is largely taken over by the machine.

An illustration of the conversational mode arises with an interactive model that has been formulated to explore ideas about the establishment of neural connections between the retina of the eye and the tectum during an animal's development. It is known that the retinal cells are eventually mapped, by their connections, in a spatially ordered manner on to the tectum and the model therefore allows the implementation of various schemes by which the ordering process may be controlled⁷. Critical assessment of particular schemes is provided by model manipulations that mimic experiments that have been carried out to perturb the developing retinotectal system by various surgical interventions. Whenever the computer is ready for a fresh instruction it types out the query 'OPTION=' and the user may then reply with a simple mnemonic option code selected from a list of some 40 alternatives. One set of codes allows the current state of the model to be examined by calling for the display of tectal mappings corresponding to various points or straight lines drawn on the retina. Other codes control the sizes and rotations of the retina, tectum and tectal grafts

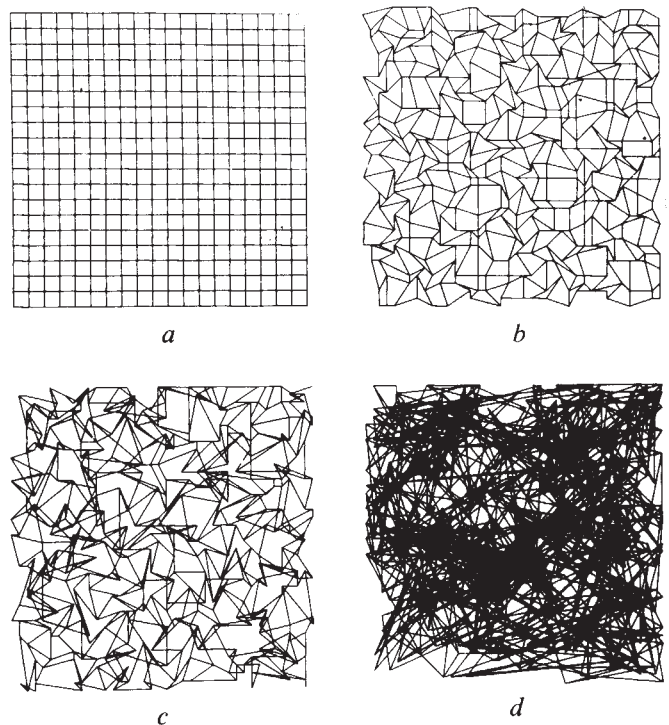


Fig. 2 One of several optional display facilities traces the rows and columns of the current retinal array as mapped on to the tectal array at any time. The result of executing an ordered mapping is shown in *a*, while *b*, *c* and *d* show the display as it appears after each retinal cell termination has been allowed a random walk on the tectum of 1, 5 and 100 steps respectively.

while the initial mapping of retinal cells on to the tectum, in either random or ordered fashion, is controlled by further codes. All option codes are checked on receipt by the computer and in the event of operator errors the user is asked to restate his chosen option; in the absence of code anomalies the computer asks for any further information, usually numerical, necessary to fulfil the particular option. This information is checked before implementation to ensure that impossible conditions, such as mapping the current retina on to too small a tectum, are not implied in the user's choice.

Further, more basic options implement changes in tectal termination positions, for example by allowing each termination the freedom of a random walk. When the random walk option is applied to an initially ordered mapping of retinal cells on to the tectum, the display of a rectangular retinal lattice mapped on to the tectum is subject to ever-increasing randomisation as shown in Fig. 2. This effect accompanies the assumption that, during development, retinal cell terminations possess an innate mobility on the tectal surface and in order to achieve the spatially ordered mapping observed in mature animals, the tectal ordering mechanisms must oppose, indeed reverse, this randomising tendency.

One scheme that has been found to order a random tectal mapping involves comparison of the retinal cell locations corresponding with neighbouring tectal terminations. Each termination is considered in turn and interchanged with a randomly selected neighbour if, and only if, its tectal position with respect to that neighbour contradicts the relative positioning of the corresponding retinal cells. In experimenting with this scheme applied to more complex model conditions some unexpected modes of behaviour have been observed. For example, when half of a tectum is rotated through 180°, so reversing its sense of direction, it was expected that a random mapping of retinal cells on to the compound tectum would

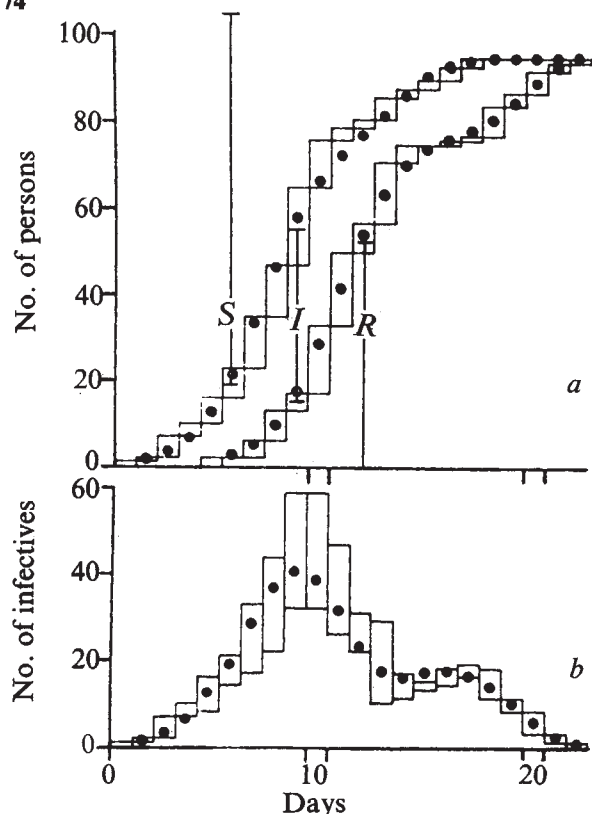


Fig. 3 The rectangles represent the maximum and minimum interpretations of field data recording the daily numbers of persons susceptible to infection (*S*), the number infected and assumed to be infectious (*I*) and the number recovered from a common cold infection as functions of time (*R*). The dots show the corresponding performance of a dynamic model evolved using interactive hybrid simulation.

eventually be ordered as if half an ordered tectum had been rotated. This idea was promptly revised when the model was observed to behave as two quasi-autonomous tecta that independently ordered their quota of randomly allocated terminations.

Dynamic systems

The eventual explanation of retinotectal mapping must have some dynamic content since changes occur in both temporal and spatial domains. In many other situations the changes that take place with time, eclipse any detailed interest in changes that occur with spatial dimensions and the geometric content of the corresponding models is reduced to the gross representation of structural features as they affect dynamic performance. This transition is highlighted in the use of compartmentation schemes, perhaps most familiar as descriptions of metabolic or drug distribution systems.

The derivation and implementation of dynamic models using computers is a subject of sufficient substance to be recognised as a separate discipline, that of computer simulation. Little of the simulation work on biological systems, however, has been carried out in sufficiently close interaction with the machine to exploit the computer simulation route to computer-aided thought. One reason for this lies in the use of high level computer simulation languages which considerably ease the burden of programming, but in execution the resultant programs are often too slow and inflexible to support on-line thinking. At least at present, custom-built applications programs remain advisable and may conveniently follow the same guidelines, and provide the same interactive facilities, as those required for the effective manipulation of purely geometric models. In terms of hardware, however, the requirements for geometric and dynamic models are not identical since many of the latter, typified by compartmental models, find their natural formulation in terms

of simultaneous differential equations. In these cases the addition of an analogue integration capability to an interactive facility can greatly enhance the speed and hence the effectiveness of communication.

The contribution that can be made by analogue integration is illustrated in an example from the field of epidemiology³. In spite of a great deal of analytical work on mathematical models of the epidemic process, the validation of even basic model assumptions has been hampered by lack of suitable field data. For such validation, accurate dynamic data are required concerning a small, but not too small, isolated, homogeneous and gregarious community into which a single infection is introduced. This requirement was apparently met when seven sets of data became available concerning common cold outbreaks on the island of Tristan da Cunha. The form of these data is shown in the upper part of Fig. 3. A flexible scheme of differential equations was therefore formulated to embrace some of the alternative ideas used in existing models of the epidemic process and the equations solved by interfacing standard analogue computer modules to perform the necessary integrations. A fast solution time (80 ms) was obtained and warranted the presentation of the solutions on a display screen concurrently with selected sets of field data. This enabled the immediate appreciation of changes in model behaviour corresponding with changes in mechanisms and values incorporated in the model structure.

A model was thus evolved that agreed well with five sets of field data but the remaining two sets remained enigmatic since experiments with the model showed that the most acceptable explanation lay in the unlikely event of a double infection involving different viruses. Since the most likely source of such viruses seemed to be the occasional visiting ship, the records of ship arrivals were examined and the occurrence of the two anomalous epidemics were found to follow the almost simultaneous presence of two visiting ships. The agreement between model and data in one of the double infection situations is shown in Fig. 3.

Present position

The essence of computer-aided thought is the effective interfacing of the conceptual prowess of man with the manipulative capability of the computer. At present, and possibly for some time to come, the finesse with which this can be accomplished is limited by available technology, but yet provides an effective means for accelerating the development of ideas. The real impediments to this use of computers in biomedical research are thus not basically technological but would seem to be more of human origin. First, the idea of computer-aided thought has not in general been brought to the attention of the biomedical researcher, at least not in a comprehensible way, so that there is little motivation to explore its application to his own work. Second, since specific ideas are to be tested, they require specific programs of some complexity in order to obtain the closeness of interaction needed for this type of application and the average biomedical researcher is rarely willing to devote the time and effort needed to acquire the necessary expertise for this purpose. Last, even where suitable facilities exist, the computer specialist is often distracted by the demands of his own subject from understanding a biomedical problem in sufficient depth to provide the researcher with an appropriate and effective means of interaction with the computer.

These problems are by no means unique, indeed they are quite familiar in other fields including that of bioengineering where it has been recognised that an engineer needs more than a superficial knowledge of biomedical subjects if he is to be maximally effective. Computer scientists specialising in biomedical applications may need to follow a similar pattern if the computer is to be fully exploited as a research tool.

We thank Dr M. Nermut for collaborating in the development of the hexon model, Mr R. A. Hope for helping develop

the retino-ectal model, Dr N. G. Wrigley for providing the electron micrograph of Fig. 1, and Dr D. A. J. Tyrell, with whom the epidemiological work was carried out.

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articles

Amplification of a specific region of the polyoma virus genome

Beverly E. Griffin & Mike Fried

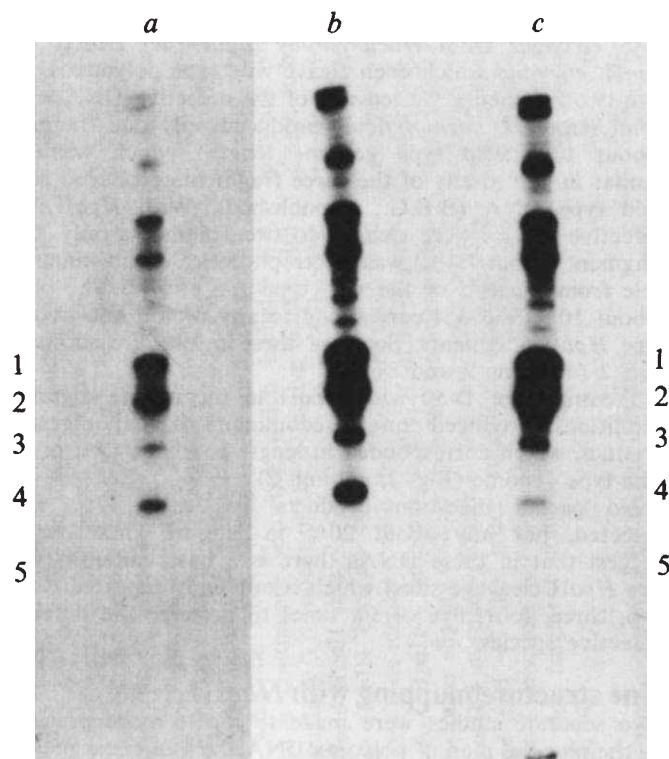
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Defective DNAs, isolated from mouse cells after infection with a clonally purified stock of defective polyoma virus, have been found to consist of nucleotide sequences from about 17% of the wild-type polyoma genome, tandemly repeated two to six times. These sequences come from the region of the viral DNA which contains the origin of DNA replication and the 5' ends of early and late stable mRNAs.

THE genome of polyoma virus is a supercoiled DNA molecule composed of about 5,200 pairs of nucleotides, and has a coding capacity for approximately 200,000 daltons of

protein. Polyoma virus can undergo at least two different types of interactions with susceptible cells. One interaction results in the death of the cell concomitant with an increase in the number of virus particles (productive or lytic infection). The other results in cells acquiring a set of characteristics which resemble those of tumour cells (non-productive or transforming infection). When cells that are capable of being lytically infected are treated with high multiplicities of polyoma virus (high virus to cell ratios), virus particles containing non-infectious supercoiled DNA molecules, heterogeneous in size, are produced. With continued high multiplicity passage, particles containing this non-infectious DNA, termed 'defective' DNA, will come to predominate the virus stock¹⁻³.

Fig. 1 Autoradiograms showing electrophoretic separation of the different ³²P-labelled DNA species obtained following infection of 3T6 mouse cells with D-50 clonal isolates. Virus stock grown from the clonal isolate containing D-50 molecules^{3,5} and wild-type helper virus, was used to infect 3T6 cells in low phosphate media in the presence of ³²P-phosphate⁶. Viral DNA was selectively extracted from infected cells¹⁵ and supercoiled DNA molecules purified by CsCl equilibrium centrifugation in the presence of EtBr (ref. 16). The band containing supercoiled DNA was isolated and the EtBr removed by extraction with CsCl-saturated 2-propanol¹⁶. DNA was recovered by alcohol precipitation after diluting the aqueous solution threefold with Tris-EDTA (10 mM-1 mM). This DNA was further separated into a number of species by electrophoresis on 1% agarose cylindrical gels (SeaKem agarose, 0.8 × 15 cm) with E buffer¹⁴. The various gel bands (1-5) were located by autoradiography, excised, and DNA eluted by electrophoresis into dialysis membranes¹⁷. The sizes of the DNAs from bands were determined by their sedimentation properties in 5-20% sucrose gradients³. By these procedures, the calculated sizes²¹ of the various DNA species were estimated to be about 34, 51, 68, 85 and 102% wild type genome length. Bands: 1, 102% defective supercoiled molecule and wild type helper supercoiled DNA; 2, 85% supercoiled defective molecules; 3, 68% supercoiled defective molecules; 4, 51% supercoiled defective molecules; 5, 34% supercoiled defective molecules. (The bands migrating more slowly than Band 1 contain linear and nicked circular forms of the different defective DNAs and various species of mitochondrial DNA.) Gels: c, Supercoiled DNA from 3T6 cells infected with the original clonal isolate³ of D-50 virus. a and b, Supercoiled DNA from 3T6 cells infected with a virus derived from purified D-50 molecules and added wild type helper viral DNA. (The D-50 molecules were purified and isolated as supercoiled species first from sucrose gradients³, followed by CsCl-EtBr density gradient centrifugation¹⁶, before being combined with the wild-type helper DNA to produce the virus stock.) a and b differ only in the length of time the gel was exposed to the X-ray film.



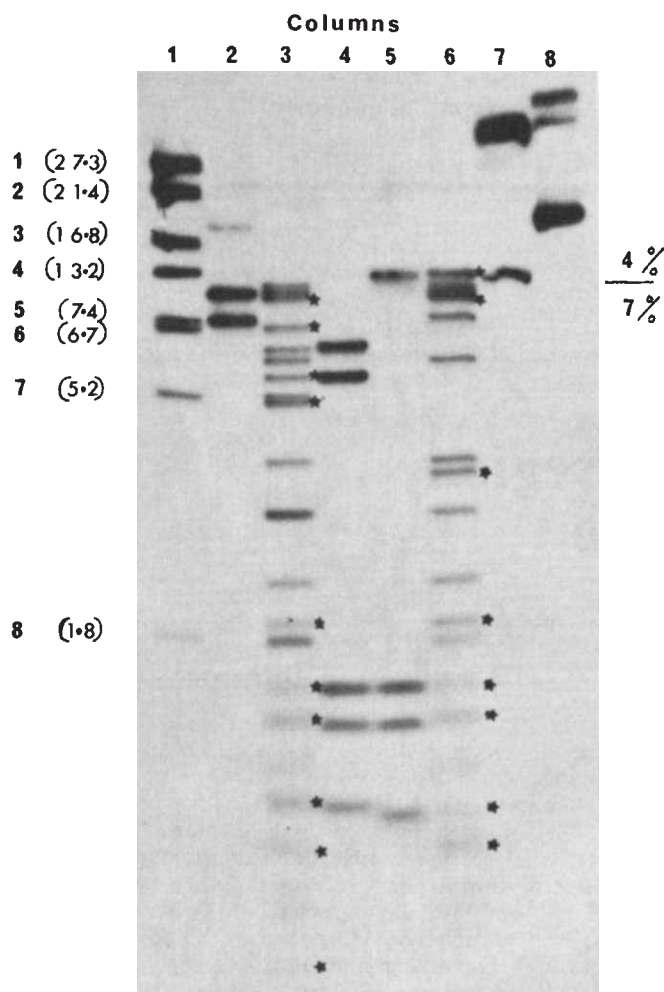


Fig. 2 Autoradiogram showing the position of restriction enzyme fragments from ^{32}P -labelled polyoma wild type (A-3 strain) DNA and D-50 DNA. Separation was accomplished by electrophoresis on a 4–7% acrylamide slab gel (20×40 cm) in Tris-borate buffer (pH 8.3). The division between the two gel concentrations is indicated. 1, Fragments 1–8 obtained after cleavage of A-3 DNA with *HpaII*; fragment sizes are given in parentheses (at left). The DNAs obtained from A-2 and A-3 polyoma virus stocks (both Pasadena large plaque strains) differ in the size of fragment *HpaII*-5. This fragment is 7.7% genome length in A-2 (ref. 6) and 7.4% in A-3 DNA⁸, the change occurring near the *HpaII* 3–5 junction (B.E.G., unpublished). D-50 and related defective DNAs have the smaller *HpaII*-5 fragment characteristic of the A-3 strain. 2, Two main fragments obtained by *HpaII* cleavage of D-50 DNA. The single product (about 17% genome length) from a limited digest is seen to migrate slightly slower than *HpaII*-3. 3, Fragments obtained after cleavage of a *HpaII*-digested sample of A-3 DNA with *HaeIII*. The position of fragments known to come from the region of the polyoma physical map which contains *HpaII*-3, -4 and -5 (B.E.G., unpublished) are indicated (*). One of the fragments from *HpaII*-5 is extremely small, and has been run off this gel. 4, Fragments obtained after cleavage of a *HpaII*-digested sample of D-50 DNA with *HaeIII*. 5, Fragments obtained after cleavage of D-50 DNA with *HaeIII*. 6, Fragments obtained after cleavage of A-3 DNA with *HaeIII*. The position of fragments known to come from the region of the polyoma physical map which contains *HpaII*-3, -4 and -5 are indicated (*). 7, Fragments 1–3 obtained after cleavage of A-3 DNA with *HhaI*. One cleavage site is known to occur in *HpaII*-2, another in *HpaII*-6 and the third in *HpaII*-5 (Fig. 4). The two large fragments (about 46% and 42%) are not clearly separated on this gel. 8, The single product (about 17% genome length) obtained after cleavage of D-50 DNA with *HhaI*. (Some partial and undigested material can also be seen.)

Most defective DNA is shorter than infectious polyoma DNA^{1–3}, contains only restricted portions of the viral genome, and in some cases may also contain host sequences

covalently linked to viral sequences⁴. Interest in the isolation of pure defective DNA populations has been stimulated by the belief that such molecules, arising as they do from situations in which selective pressures are being exerted within cells, would be extremely useful both for studying virus–cell interactions and the evolution of the viral genome. A number of different defective molecules have recently been cloned in the presence of infectious wild-type polyoma (helper) molecules⁵. Molecules about 50% (designated D-50) the size of infectious polyoma DNA were purified from one clonal isolate and were found by heteroduplex studies to be homogeneous in size and to contain a single region of homology to about 18% of the sequences in polyoma DNA⁵.

This report describes the sequences present in D-50, shows that other defective molecules present in the D-50 preparation contain the same sequences, and relates these sequences to those found around the origin of DNA replication in wild-type polyoma DNA.

Characterisation of defective species

A population of DNA molecules isolated from cells infected with a virus stock containing both polyoma D-50 molecules and wild type helper virus was found to contain molecules corresponding to about 34, 51, 68, 85 and 102% the size of the wild type polyoma genome (Fig. 1). The 51% species was found to be identical with the D-50 DNA previously analysed by electron microscopy⁵. The designation D-50 for this species will therefore continue to be used in this report. Heteroduplex studies showed the region of homology between D-50 and wild type DNA molecules to begin on the latter about 16% from the *Escherichia coli* RI restriction enzyme (*EcoRI*) single cleavage site⁵. On the physical map of polyoma DNA this site can lie in either restriction fragment *HpaII*-6 or *HpaII*-4 (ref. 6), (Fig. 4). To locate the homology region on the polyoma physical map and define the D-50 and related defective species more precisely, a restriction enzyme analysis was performed.

Cleavage with restriction enzymes

The resistance of D-50 molecules to cleavage by *EcoRI* restriction enzyme was shown earlier^{5,6}. The other defective species from the same virus stock were also resistant to *EcoRI*. Moreover, all the species were resistant to cleavage with enzymes from *Haemophilus influenzae*, *HinIII* and *HinII*, enzymes which each cleave wild type polyoma DNA into two fragments^{6,7}. Cleavage of the defective DNAs with *HhaI* (from *H. haemolyticus*) produced only one fragment (about 17% wild type genome length) which was not similar in size to any of the three fragments produced from wild type DNA (B.E.G., unpublished). With *HpaII*, the defective species were cleaved to two fragments only. One fragment (about 7.4%) was electrophoretically indistinguishable from *HpaII*-5 of the wild type A-3 strain⁸. The other (about 10%) did not correspond to any of the known wild type *HpaII* fragments. Some of these results are shown in Fig. 2 (columns 2 and 8).

Treatment of D-50 with *HpaII* in incomplete digestion conditions produced one predominant partial digestion product, which corresponded in length to about 17% of the wild-type genome (Fig. 2, column 2).

No partial digestion products less than 17% were detected, nor any about 20% in length. These results suggest that in these DNAs there is a basic subunit (with two *HpaII* cleavage sites) which is tandemly repeated either two, three, four, five or six times to produce the different defective species.

Fine structure mapping with *HaeIII*

Two separate studies were made to locate more precisely on the physical map of polyoma DNA the sequences present in the defective molecules. In one, the defective DNAs and

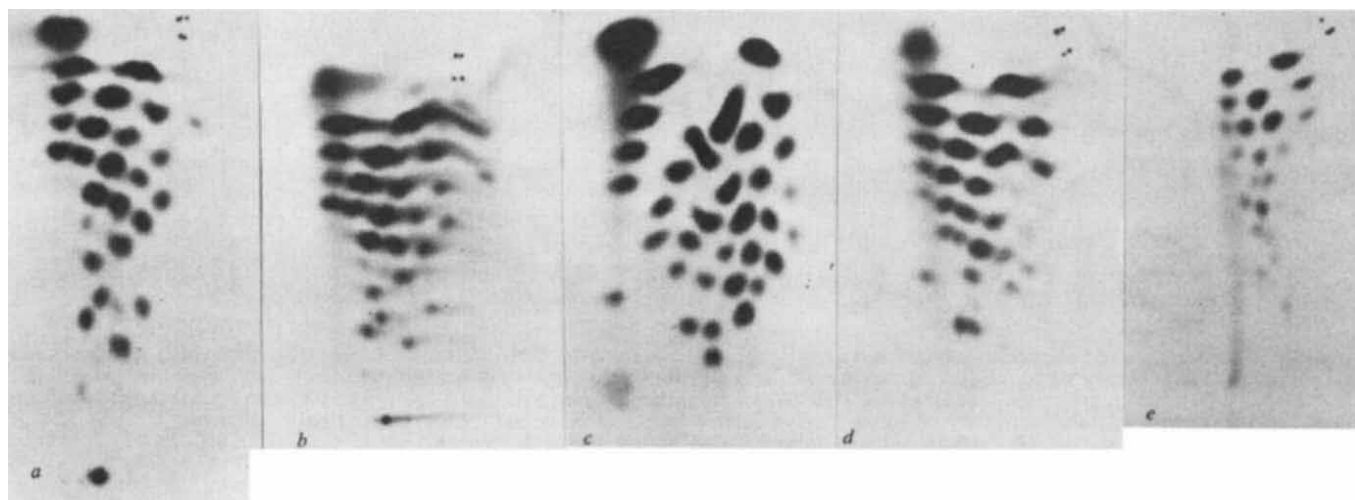


Fig. 3 Autoradiograms of the two-dimensional fractional separation of pyrimidine tracts of some depurinated ^{32}P -labelled *HpaII* fragments from wild type polyoma (A-3 strain) DNA and D-50 DNA. Depurinations and separations were carried out as described previously⁹: the first dimensional separation (from right to left) was carried out by electrophoresis on cellulose acetate strips at pH 3.5, and the second dimensional separation (from bottom to top) by homochromatography using homomix C¹⁸. Fractionation of pyrimidine tracts from the *HpaII*-5 fragment from A-3 DNA (a), and the 7.4% *HpaII* fragment from D-50 DNA (b). a and b can be seen, at least qualitatively, to be identical. Fractionation of pyrimidine tracts from the *HpaII*-3 fragment from A-3 DNA (c), the *HpaII*-4 fragment from A-3 DNA (e) and the 10% *HpaII* fragment from D-50 DNA (d). All pyrimidine tracts present in d can be found in either c or e, although many tracts present in c and e, or in the entire wild type genome (not shown), are not found in d. Three large oligonucleotides (including oligo (T₇ or ₈), marked* in c) are characteristic of *HpaII*-3 (B.E.G., unpublished) and can be seen in the D-50 fragment (d). The isoplith C₃T₃, marked (*) in e, is present in high yield both in *HpaII*-4 and d.

their *HpaII* cleavage products were treated with a restriction enzyme from *H. aegyptius* (*HaeIII*) which cleaves polyoma wild-type DNA into approximately 23 fragments. The relationship between the *HaeIII* fragments and the *HpaII* fragments (used to construct the physical map of the polyoma DNA⁶) are known (B.E.G., unpublished). Therefore, *HaeIII* can be used as an effective probe into the composition of the defective molecules. The cleavage by *HaeIII* alone (Fig. 2, column 5) of the defective species gave one large fragment (about 13% wild type genome length) together with three small fragments, two of which could be identified as coming from *HpaII*-3 and one from *HpaII*-5. In wild-type DNA, these three small fragments join to form a continuous sequence which includes the *HpaII* 3-5 junction. Treatment of the defective DNAs with both *HaeIII* and *HpaII* (Fig. 2, column 4) showed that the larger (13%) *HaeIII* product could be cleaved into two fragments. One was identical with the fragment from wild type strain A-3 *HpaII*-5 (adjacent to the *HpaII* 4-5 junction) and the other slightly smaller than the fragment from wild-type *HpaII*-4 normally found at the 4-5 junction.

Since one end of the latter fragment must lie at the *HpaII* 4-5 junction and the other at the *HaeIII* site in *HpaII*-3, this fragment contains sequences from both *HpaII*-3 and -4.

Fine structure mapping by depurination

Depurination fingerprints⁹ of D-50, and the 85 and 102% species were made and found to be identical, thus supporting the relationship between these species. They were considerably less complicated than a depurination fingerprint of wild-type polyoma DNA. Pyrimidine tracts characteristic of wild type *HpaII* fragments 1, 2, 6, 7 and 8 were found to be absent (B.E.G., unpublished). No pyrimidine tracts were found in the defective species which were not present in wild-type polyoma DNA.

In a second study, a comparison of the depurination fingerprint of the smaller *HpaII* fragment from D-50 (about 7.4% wild type genome length) with *HpaII*-5 from wild

type DNA showed the two to be identical. The fingerprint of the larger *HpaII* fragment (about 10% genome length) showed pyrimidine tracts characteristic of both wild type fragments *HpaII*-3 and -4 (Fig. 3).

All the results are consistent with the defective DNAs being composed of tandemly repeating 17% units made up of all the viral sequences from polyoma *HpaII*-5 (wild type A-3 strain) and part of the sequences from *HpaII*-3 and -4. The results of the study on D-50 are summarised on a physical map, relative to wild type DNA⁶, in Fig. 4.

Conclusions

One of the spontaneously arising defective species from polyoma virus has been cloned³ and is here studied in detail. It has been shown to be made up of molecules containing two- to sixfold tandem repeats of a segment of approximately 17% of viral DNA. Restriction enzyme analysis, especially with *HaeIII*, has defined the viral sequences in the 17% subunit as extending from *HpaII*-3 through *HpaII*-5 into *HpaII*-4 on the polyoma physical map⁶. One of the *HaeIII* fragments from the defective molecules is not identical with any of the wild type *HaeIII* fragments and represents a fusion of sequences from *HpaII*-3 and *HpaII*-4. From these data the exact location of the ends of the defective subunit cannot be precisely placed on the physical map. The correlation of these studies with the size of the homology region observed by Roberson and Fried⁵ suggests that most if not all of the sequences in the subunit are viral. Assuming only viral sequences to be present and knowing the order and approximate size of the *HaeIII* fragments of the wild type DNA in this region it can be estimated that of the sequences in the 17% subunit, 18-27% come from *HpaII*-3 and 42-53% from *HpaII*-4. The results on the size of the defective subunit and its position on the physical map of polyoma DNA are in good agreement with the previous heteroduplex studies⁵.

The segment of viral DNA present in D-50 and related defectives contains two of the biologically interesting markers so far located on the physical map of polyoma

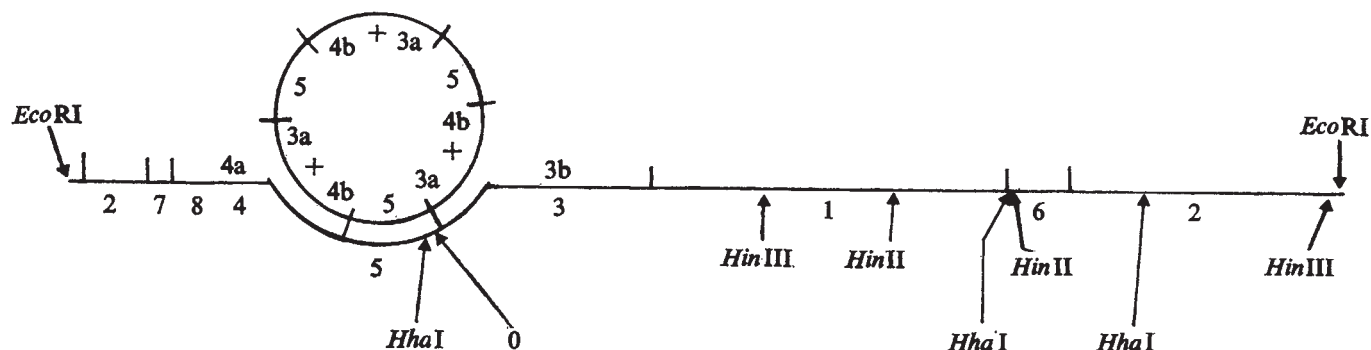


Fig. 4 Diagrammatic representation of the homology region between D-50 DNA (circle) and *EcoRI*-treated wild type polyoma DNA (linear). Homology begins $16 \pm 2\%$ from the left end of wild-type DNA and extends to about 34% from the same end⁸. Numbers 1-8 on linear map show the order of the eight *HpaII* fragments of polyoma DNA⁶, and 5 and 3a+4b (circle) the order of the two *HpaII* fragments of D-50 (and the defective DNAs related to it, as described in the text). *HpaII* fragments 5 (about 7.4%) and 3a+4b (about 10%) make up the subunit (about 17%) found tandemly repeated three times in D-50 DNA, and two, four, five and six times in the other defective DNA related to D-50. The approximate site of the origin of DNA replication (O) is indicated⁶; note that this site is repeated three times in D-50 DNA. The cleavage sites of three other restriction enzymes, *HinIII*⁴, *HinII* and *HhaI* (B.E.G. unpublished) are indicated on the physical map of polyoma DNA.

DNA. One is the origin of DNA replication, which has been mapped around the *HpaII* 3-5 junction at 71 ± 3 map units^{6,19}. The other contains the 5' ends of the early and late stable RNAs (transcribed from different DNA strands) which have been mapped in *HpaII*-5 (ref. 10). It is interesting to note that the regions in which viral DNA synthesis terminates^{6,19} and where the 3' ends of viral messengers have been mapped¹⁰, have not been detected in these defective DNAs.

Molecules containing multiple origins of DNA synthesis might be expected to have selective advantages for replication over those molecules with a single origin. When apparently defective-free virus is grown at high multiplicity for a single cycle, defectives similar to those described here arise with high frequency. Although the size of the basic subunit seems to be variable, in most cases it seems to contain *HpaII*-5 and thus can be detected by greater than unimolar equivalents of this fragment in the DNA⁶. Another clonal isolate containing molecules about 47% (D-47)⁶ of the viral genome seems to contain a repeating unit of a 16% segment of viral DNA which includes *HpaII*-5 (B.E.G. and M.F., unpublished). Similar molecules with repeated segments of sequences which contain the origin of DNA replication have also been detected in SV-40 (ref. 12) and PML virus^{11,20} passaged at high multiplicities. The detection of repeated viral sequences in *EcoRI* resistant defective molecules from uncloned polyoma DNA preparations derived from high multiplicity passage may also contain repeats in this region¹³. Note that the type of defective defined here seems to be only one from among several types of defective DNAs that arise after passage of polyoma virus at high multiplicity.

It is not clear at present how D-50 and related DNAs arise. There are a number of mechanisms by which these defective DNAs can be generated. One possibility is that they are formed by errors in replication⁵. The study of other cloned defectives with basic subunits of different sizes will help clarify their origin. For instance, if the midpoint of all the different defective subunits were the same it would suggest that DNA synthesis proceeds bidirectionally at equal rates before the subunit is excised and ligated. On the other hand, if one end of the defective basic subunit were always at the same position on the physical map whereas the other end varied in position then unidirectional replication for various lengths before excision and ligation might be suggested.

In this study, a correlation between the quantity of any species of defective DNA in the clonal isolate and the number of tandem repeats of the 17% subunit in that species has been observed (Fig. 1). Two explanations may

account for the greater abundance of the larger defective species. One would be the greater selective advantage for replication available to molecules with more origins of DNA synthesis. The other would be the possible difficulty of stable encapsidation (necessary for continued passage of the particular species) of the smaller DNA molecules.

The defective molecules containing multiple tandem sequence repeats are presumably derived from a common precursor of about 17% of the polyoma genome. We have not been successful in isolating this postulated small precursor molecule, but it would not be expected to be present in great quantities for reasons given above. The DNA isolation procedure used would also tend to discriminate against such a low molecular weight molecule. One of the interesting results from the present study is, however, the apparent uniformity of the repeating subunit. The results from a number of experiments suggest that there is little or no variation in the size of the *HpaII* 7.4% fragment and if there is any variation in the size of the 10% *HpaII* fragment, it is outside the scope of detection of the methods used. This suggests that D-50 and related molecules are derived from the subunit by recombination events.

Further, it is interesting to note that DNA derived from virus stocks produced from purified D-50 molecules and added helper DNA generated all the species of the multiple repeat defectives found in the parental population (Fig. 1). This generation of both larger and smaller related defective molecules from the D-50 species suggests that intramolecular and possibly intermolecular recombination processes can occur. Moreover, in the DNA produced from virus stocks made from the purified D-50 molecules the D-50 species is seen to be present in greater yields than the 68% species (Fig. 1a and b), whereas in the parental population the reverse is true (Fig. 1c). This seems to mitigate against the rolling circle model of DNA replication²² as a mechanism for the generation of the related defectives, as this type of replication would not show any preference for the production of D-50 species over the 68% species.

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Implications for theories of anaesthesia of antagonism between anaesthetic and non-anaesthetic steroids

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The non-anaesthetic steroid $\Delta 16$ -alphaxalone antagonises the depressant action of the anaesthetic steroid alphaxalone on synaptic transmission. This antagonism is not predicted by classical theories of anaesthesia and therefore poses fundamental questions about the molecular basis of anaesthesia.

It is now widely accepted that there is a good correlation between the anaesthetic potency of a substance and its lipid solubility¹⁻⁴. This correlation has led to a number of hypotheses of anaesthetic action, notably the Meyer-Overton rule of lipid solubility⁵ and the critical volume hypothesis of Mullins⁶. These hypotheses state that anaesthesia occurs whenever a certain critical concentration or a critical molar volume of an inert substance is achieved in the neuronal membranes. These theories have a serious weakness, however, in that they concentrate attention on substances that are both lipid-soluble and anaesthetic, and ignore those that are lipid-soluble but have no anaesthetic properties.

Some steroids possess anaesthetic properties whereas others do not⁷. Systematic examination of some 5 α -pregnanes has shown that steroids having the same functional groups could be either anaesthetic or non-anaesthetic according to their precise structure^{8,9}. These observations are not easily reconciled with the hypotheses of anaesthetic action based on lipid solubility, as the steroids are themselves lipids. It could be argued that the non-anaesthetic steroids fail to produce general anaesthesia simply because they do not reach the brain in sufficient quantity to cause anaesthesia. It follows from this argument that, if we could apply 'non-anaesthetic' steroids directly to the nerve cells of the brain, we should be able to reveal their anaesthetic properties.

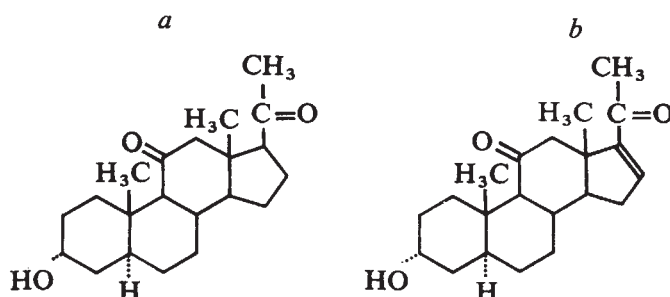
One preparation suitable for testing this idea is the isolated olfactory cortex of the guinea pig^{10,11}. A number of general anaesthetics depress excitatory synaptic transmission in this preparation at concentrations likely to be found in the brain during anaesthesia¹²⁻¹⁵. If we could show that anaesthetic steroids also depressed excitatory synaptic transmission in this preparation, and that non-anaesthetic steroids did not, then we could conclude that either the non-anaesthetic steroids cannot penetrate synaptic membranes or that they can, but fail to alter normal membrane function. In either event some factor in addition to lipid solubility would be required to explain such results.

In the work presented here, we have examined the pharmacological action of two closely related steroids on synaptic transmission in the olfactory cortex. One of the compounds,

3 α -hydroxy-5 α -pregnane-11,20-dione (alphaxalone) is a powerful anaesthetic and is the main active component in the clinically used anaesthetic Althesin. The other, 3 α -hydroxy-5 α -pregnane-16-ene-11,20-dione ($\Delta 16$ -alphaxalone) is non-anaesthetic⁹. The structures of these two substances (Fig. 1) differ only by a double bond in the D ring of the steroid nucleus.

Isolated preparations of guinea pig olfactory cortex have been shown to generate stable and characteristic field potentials in response to electrical stimulation of the lateral olfactory tract (LOT)¹¹⁻¹⁵. If a recording electrode is placed on the pial surface of the prepiriform cortex close to the LOT and a pulse is subsequently applied to the anterior end of the LOT, a complex wave form can be recorded (see Fig. 2). This comprises an initial wave—the compound action potential caused by activity in the LOT fibres—followed by a negative wave (the population excitatory postsynaptic potential, or population e.p.s.p.) which has been identified as the field potential arising from the synchronous depolarisation of the apical dendrites of many of the cortical neurones. If the population e.p.s.p. is sufficiently large, a number of positive peaks may be superimposed on it. These peaks reflect the synchronous discharge of the cortical cells in response to the population e.p.s.p. and are called population spikes¹⁷ (see Fig. 2). (If the recording electrode is placed in the pyramidal cell layer instead of on the cortical surface, a similar potential can be recorded, but of reversed polarity¹⁸ (Fig. 3).) The amplitude of the population e.p.s.p. is proportional to the number of active synapses and to the degree of depolarisation of each postsynaptic site; the area of the population spikes is proportional to the number of discharging cortical cells. In this paper we have taken the amplitude

Fig. 1 Structural formulae of steroids used. a, Alphaxalone; b, $\Delta 16$ -alphaxalone.



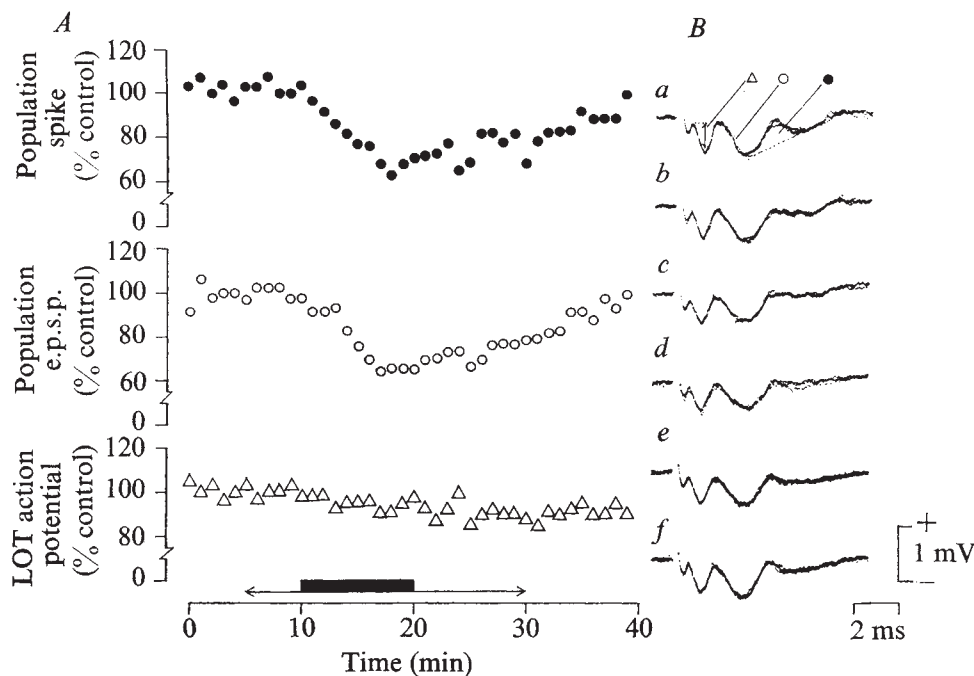


Fig. 2 Depressant action of alphaxalone on the evoked potentials of the olfactory cortex. **A**, Time course of experiment. Arrowed line under black bar indicates period during which CSF containing liposomes was superfusing the preparation. Black bar indicates period of exposure to alphaxalone (5×10^{-5} M). **B**, Sample records of four to six superimposed sweeps taken at various times during the experiment. **a**, Control; **b**, 6 min in liposomes; **c**, 5 min in alphaxalone; **d**, 10 min in alphaxalone; **e**, 10 min after alphaxalone; **f**, recovery. Alphaxalone was administered as the steroid component of liposomes. Egg yolk lecithin was dissolved in chloroform-methanol (2:1 v/v) together with the steroid in a molar ratio of 1:1. The solutions were evaporated to dryness under nitrogen and kept under vacuum (10^{-3} mmHg) for 1–4 h to remove residual traces of solvent. Artificial CSF (refs 12–14) was then added to yield a final concentration of 50 μ M phospholipid. Solutions at 4°C were then subjected to ultrasonic dispersion for 2–5 min using an MSE probe sonicator to suspend the phospholipid as small vesicles.

of the population e.p.s.p. at a fixed latency from the stimulus as our chief measure of postsynaptic activity^{12–15}.

As steroids are very hydrophobic substances, they are very sparingly soluble in water. Clinically, they are administered as micelles with Cremaphor EL (a polyoxyethylated castor oil) as the carrier¹⁶ but Cremaphor was toxic to our isolated preparations. To overcome this, we applied alphaxalone as the steroid component of lecithin vesicles (liposomes) suspended in the artificial cerebrospinal fluid (CSF) that superfused the isolated cortical tissue. This method of applying the steroids has proved quite satisfactory.

When liposomes containing cholesterol, or lacking a steroid component, were added to the artificial CSF that superfused the isolated cortical tissue, little change was seen in the evoked potentials (Figs 2 and 3). If the superfusing artificial CSF containing lecithin liposomes was changed for one containing liposomes with alphaxalone in a 1:1 molar ratio with the phospholipid (to give a final concentration of 5×10^{-5} M alphaxalone in the artificial CSF) then the population e.p.s.p. and population spikes became depressed but the LOT compound action potential was not affected (Fig. 2). Provided the tissue was not exposed to the alphaxalone for long periods (greater than 20 min) the depression of the population e.p.s.p. and population spike rapidly reversed when artificial CSF containing lecithin liposomes replaced that containing alphaxalone-loaded liposomes. Thus, alphaxalone depressed the population e.p.s.p. and population spikes without blocking nerve impulse conduction in the afferent LOT fibres. The depression of the population e.p.s.p., however, produced by a given dose of alphaxalone varied rather widely, in the range 25–100% for a concentration of 5×10^{-5} M in the CSF, although it generally lay between 30 and 60% (Table 1). As with other general anaesthetics^{12–15} this depression occurred at concentrations of alphaxalone likely to be found in the brain during anaesthesia. (An anaesthetic dose of alphaxalone has been found to be approximately 3 mg kg⁻¹ for a number of species¹⁶. As the anaesthetic which reach the brain cells by way of the extracellular fluid which accounts for 20–30% of the total body weight¹⁹, 3 mg kg⁻¹ alphaxalone given intravenously will have a maximum concentration in the extracellular space of 3×10^{-5} to 5×10^{-5} M (ref. 12).)

If the liposomes superfusing the tissue contained not alphaxalone but $\Delta 16$ -alphaxalone, a very much smaller depression of

the population e.p.s.p. of about 10–15% was seen (Table 1). Furthermore, increasing the duration of the superfusion by $\Delta 16$ -alphaxalone beyond the normal 10 min period (up to a maximum of 45 min) did not further increase the depression of the population e.p.s.p.

These results correlate well with the effects of these steroids *in vivo*; the anaesthetic steroid alphaxalone depresses the population e.p.s.p. by 30–60%, whereas the non-anaesthetic steroid $\Delta 16$ -alphaxalone has little depressant action. These results suggest that both steroids can penetrate the synaptic membranes, as they both depress the population e.p.s.p., but, unlike alphaxalone, $\Delta 16$ -alphaxalone is not able to disrupt synaptic transmission to any great extent.

Antagonism of depressant action of alphaxalone by $\Delta 16$ -alphaxalone

After an experiment in which the depressant action of $\Delta 16$ -alphaxalone had been tested, we exposed the same preparation to alphaxalone and discovered that alphaxalone had only a slight depressant action. The preparation was left in artificial CSF for 3 h and was then exposed again to alphaxalone. After this time the preparation regained its sensitivity to alphaxalone (Fig. 3). This antagonism of the depressant action of alphaxalone by $\Delta 16$ -alphaxalone has now been confirmed in ten separate experiments using two different samples of alphaxalone and $\Delta 16$ -alphaxalone, and three different samples of lecithin (Table 1). Of the ten experiments, four showed almost complete reversal of the $\Delta 16$ -alphaxalone block 1–3 h after exposure to $\Delta 16$ -alphaxalone, two showed a partial reversal and in the remaining four experiments, the depressant action of alphaxalone was blocked throughout the experiment (up to 3 h).

If a preparation had first been exposed to alphaxalone before treatment with $\Delta 16$ -alphaxalone, subsequent exposures to alphaxalone generally caused the same depression of the e.p.s.p. as that seen initially. To reveal the antagonism of the depressant action of alphaxalone by $\Delta 16$ -alphaxalone it was necessary to expose the tissue to $\Delta 16$ -alphaxalone—before making any test for the depressant action of alphaxalone. For this reason the depressant action of the alphaxalone-containing liposomes was checked in a parallel experiment on a preparation not pretreated with $\Delta 16$ -alphaxalone. Both the control experiments and those demonstrating the antagonism were performed on the same day to eliminate variation in the release of alphaxalone

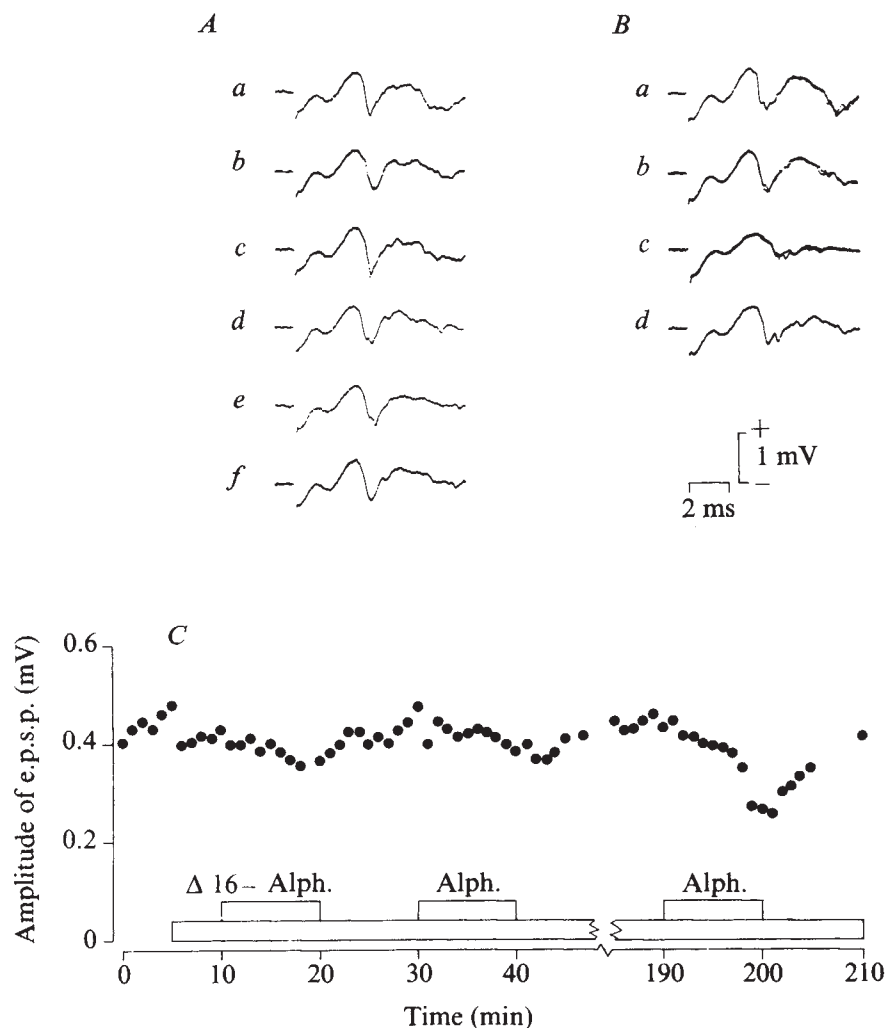


Fig. 3 Action of $\Delta 16$ -alphaxalone on evoked potentials of olfactory cortex. *A* and *B*, Sample records taken from the cell body region of the slice at various times during the experiment shown below. *A*, Records taken in the first 45 min of the experiment. *a*, Control; *b*, $\Delta 16$ -alphaxalone; *c*, control 2; *d*, 5 min in alphaxalone; *e*, 10 min in alphaxalone; *f*, recovery. *B*, Records taken after 185 min. *a*, Control 3; *b*, 5 min in alphaxalone; *c*, 10 min in alphaxalone; *d*, recovery. *C*, Time course of the experiment. $\Delta 16$ -alphaxalone (5×10^{-5} M) had little depressant effect. Initial exposure to alphaxalone (5×10^{-5} M) showed only slight depressant activity but a subsequent exposure to alphaxalone (approximately 3 h after the exposure to $\Delta 16$ -alphaxalone) markedly depressed the population e.p.s.p. Lecithin (10^{-4} M) in CSF after 5 min for duration of experiment.

from liposomes as a result of ageing or the formation of lysolipids. One example of these paired experiments can be seen in Fig. 4.

Nature of the antagonism

The antagonism of the depressant action of alphaxalone by $\Delta 16$ -alphaxalone could be the result of one of two processes either $\Delta 16$ -alphaxalone prevents the release of alphaxalone from the liposomes (that is, it is an artefact of the method of application) or it antagonises the depressant action of alphaxalone on some process involved in synaptic transmission.

As the antagonistic effects of $\Delta 16$ -alphaxalone on the depression of the e.p.s.p. by alphaxalone could persist for several hours, it seems unlikely that small amounts of $\Delta 16$ -alphaxalone were merely reducing the release of alphaxalone from the phospholipid vesicles. This argument is further supported by the observation that, following a short exposure (5 min) to $\Delta 16$ -alphaxalone, one preparation did not exhibit resistance to the

depressant action of alphaxalone. Moreover, the antagonism did not develop if a preparation had first been exposed to alphaxalone before treatment with $\Delta 16$ -alphaxalone. These observations strongly suggest that the antagonism is a genuine pharmacological event.

Given that $\Delta 16$ -alphaxalone antagonises the depressant action of alphaxalone by some biological mechanism, there are four ways in which this could be brought about. First, $\Delta 16$ -alphaxalone could prevent alphaxalone penetrating the synaptic membranes. Second, $\Delta 16$ -alphaxalone could be acting as a competitive antagonist at some specific site or sites. Third, as synaptic transmission is a multistage process, $\Delta 16$ -alphaxalone and alphaxalone could act at different sites but have opposing actions which tend to cancel. Fourth, $\Delta 16$ -alphaxalone could desensitise the preparation to alphaxalone.

Of these four possibilities, the first two seem the most plausible but we have no evidence that enables us to distinguish between them. The third possibility seems unlikely because

Table 1 Depression of population e.p.s.p. at the end of a 10-min exposure to 50 μ M alphaxalone or 50 μ M $\Delta 16$ -alphaxalone

Drug	Pretreatment	Depression of population e.p.s.p. (% control)			No. of experiments
		Mean	$\pm$ s.e.m.	Range	
$\Delta 16$ -alphaxalone	None	14.8	$\pm$ 2.5%	0-25%	9
Alphaxalone	None	45.7	$\pm$ 5.7%	25-100%	12
Alphaxalone (10 min after pretreatment)	(20 min in $\Delta 16$ -alphaxalone)	9.4	$\pm$ 3.26%	0-35%	10

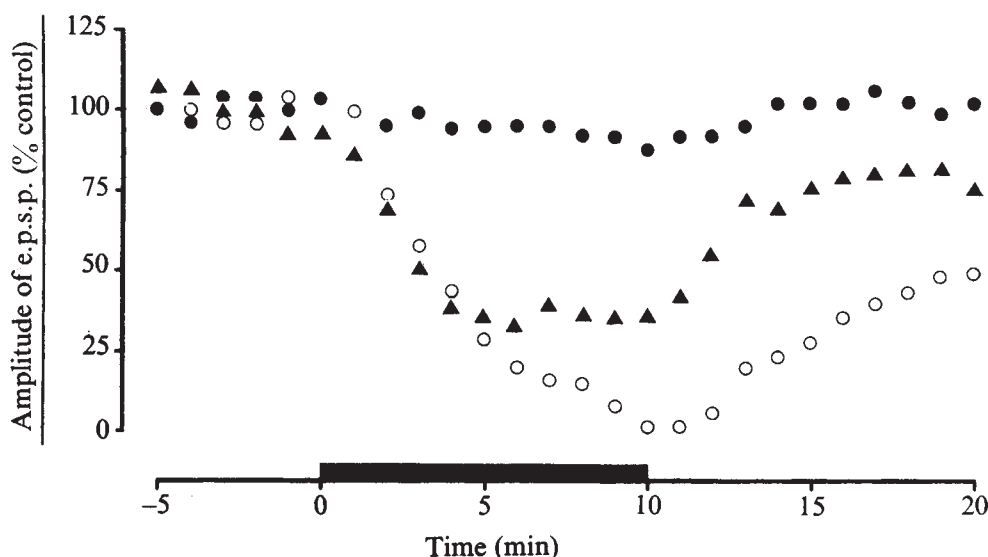


Fig. 4 Example of blocking of alphaxalone action by $\Delta 16$ -alphaxalone and its subsequent recovery compared with the depression of the e.p.s.p. produced in a preparation not pretreated with $\Delta 16$ -alphaxalone. $\circ$, Depression produced in an untreated preparation; $\bullet$, block of alphaxalone-induced depression 12 min after exposure to $\Delta 16$ -alphaxalone; $\blacktriangle$, recovery of sensitivity to alphaxalone 160 min after exposure to $\Delta 16$ -alphaxalone. Bar, alphaxalone (5×10^{-5} M) exposure.

$\Delta 16$ -alphaxalone and alphaxalone show qualitatively similar effects on the synaptic potentials. Furthermore, the antagonism outlasts the depressant action of $\Delta 16$ -alphaxalone (Fig. 3). The fourth possibility is also unlikely as $\Delta 16$ -alphaxalone does not antagonise the depressant action of alphaxalone in preparations which had been exposed to alphaxalone before treatment with $\Delta 16$ -alphaxalone. Further indirect evidence against a desensitisation hypothesis comes from experiments on mice, in which it has been shown that successive injections of alphaxalone result in a slight increase in the sleep time resulting from a given dose of anaesthetic²⁰.

It is possible that the antagonism between these steroids reflects the existence of specific anaesthetic receptors for steroids, especially as the structure-activity relationships for anaesthetic potency are so well defined^{8,9}. No firm answer can however, be given at present, but it should be noted that both 5α - and 5β -pregnanes may be anaesthetic in spite of the marked difference in the shape of the steroid nucleus in these two series. Furthermore, steroids are very lipid-soluble and show some of the nonspecific membrane effects of other general anaesthetics⁴—for example, they protect erythrocytes from lysis²¹, presumably by expanding the membranes⁴.

Implications for theories of anaesthesia

Hypotheses of anaesthetic action based on simple solubility models, such as the Meyer-Overton lipid solubility rule of anaesthesia⁵ or the critical volume hypothesis of Mullins⁶, imply that all anaesthetics act in the same region. This has led to the formulation of the unitary hypothesis of anaesthetic action, in which it is assumed that general anaesthetics produce their effects by simple physicochemical interactions with hydrophobic regions of the cellular membranes, and not by interaction with specific sites²². It is implicit in all three hypotheses that the effects of two lipophilic compounds should be synergistic, not antagonistic²³, and this has indeed been shown for several pairs of compounds^{24,25}. One striking example is the synergism of tetrahydrocannabinol and diethyl ether. Although tetrahydrocannabinol is highly lipophilic it is not an anaesthetic, yet when given intravenously to mice it reduces the amount of ether required to abolish the righting reflex²⁶.

One hypothesis of anaesthetic action does predict the existence of antagonism between different classes of anaesthetic. This is the degenerate perturbation hypothesis proposed by Metcalfe *et al.*²⁷ to account for the local anaesthetic action of a wide variety of organic compounds. This hypothesis is based on the opposing actions, on model membrane systems, of small molecules such as benzyl alcohol and large rigid molecules, such as androstane; benzyl alcohol increases and androstane decreases the fluidity of phospholipid bilayers²⁸⁻³⁰. Although

clinical concentrations of some general anaesthetics (halothane and methoxyflurane) have been shown to fluidise phospholipid bilayers³¹, preliminary results with electron spin resonance techniques have shown no change in membrane fluidity when either alphaxalone or $\Delta 16$ -alphaxalone are added to a phospholipid bilayer (T.R.H., unpublished). This suggests that the antagonism of the depressant action of alphaxalone by $\Delta 16$ -alphaxalone is not mediated by generalised perturbations of membrane structure and presumably depends on more specific interactions within the synaptic membranes.

The antagonism of the effects of alphaxalone by $\Delta 16$ -alphaxalone cannot be accommodated by any of the hypotheses of anaesthetic action mentioned above and we must, therefore, conclude that they do not apply to anaesthesia induced by steroids. It remains to be seen whether substances other than $\Delta 16$ -alphaxalone can antagonise the depressant action of alphaxalone and whether $\Delta 16$ -alphaxalone can antagonise the action of other general anaesthetics on synaptic transmission.

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Isolation of polar insertion mutants and the direction of transcription of ribosomal protein genes in *E. coli*

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Transcription of ribosomal protein genes in the str-spc region in Escherichia coli seems to be exclusively anti-clockwise. Insertion mutations in this region inactivate many but not all distal ribosomal protein genes. Thus, there seems to be more than one transcriptional unit for the ribosomal protein genes in this region.

MANY ribosomal protein (r-protein) genes in *Escherichia coli* are clustered at the *str-spc* region of the chromosome (see ref. 1 for review). Little is known about the organisation of these genes, however, or the mechanism of the regulation of their expression. Recent isolation of λ transducing phages carrying many r-protein genes from this region² has made it possible to study these problems in detail. Here we report the isolation of insertion mutations of the transducing phage λ spc1 that inactivate the expression of many, but not all, of the r-protein genes carried by this phage. In this connection, we have reinvestigated the direction of transcription of r-protein genes in the *str-spc* region. We have found that, contrary to the previous suggestion³, the direction of transcription is anti-clockwise on the *E. coli* genetic map. The inactivated genes in our mutants are distal to the sites of the insertion mutations, and may belong to a single transcriptional unit.

Insertion mutations affecting r-protein genes

The λ spc1 phage carries a *spc*^s gene as well as *aroE*⁺ and *trkA*⁺ genes². Bacterial strains carrying the *spc*^r allele become spectinomycin-sensitive (Spc-S) when they are lysogenised with λ spc1, because *spc*^s is dominant over the *spc*^r allele²¹. The lysogen from which we isolated Spc-R mutants had the following relevant chromosomal genetic markers: *KdpABC5*, *trkA401*, *spc*^r, *str*^r, *fus*^r, *recA*; and carried λ spc1 and a helper phage λ c1857S7. Thus this strain is diploid for the *spc* gene (*spc*^s/*spc*^r) and is phenotypically Spc-S. The selection of Spc-R mutants was done on tryptone-yeast extract agar plates containing Spc (100 μ g ml⁻¹). This medium has a low concentration of potassium and does not enable growth of *trkA* mutants. Thus Spc-R colonies selected on this medium must have retained the *trkA*⁺ gene carried by the λ spc1 phage. The Spc-R phenotype presumably resulted from some mutation that inactivated or altered the *spc*^s gene on the phage.

Transducing phages were then isolated from these Spc-R mutants and characterised. Out of 20 independently isolated Spc-R colonies the transducing phage could be recovered from 18, and 14 of them carried an insertion of a small piece of DNA in the phage (see below). Thus the inactivation of the *spc*^s gene in these 14 mutants seemed to be a result of an insertion. Two such mutants, λ spc1-I15 and λ spc1-I16, were studied in detail.

To examine the expression of other r-protein genes carried

by these mutant phages, we have measured the stimulation of r-protein synthesis in ultraviolet-irradiated *E. coli* cells after infection by the mutant phages and compared the stimulation with that caused by the parental transducing phage λ spc1 (Table 1).

The parent λ spc1 stimulates the synthesis of about 20 r-proteins (ref. 2 and Table 1). The strong stimulation of the synthesis of proteins S4, S11, S13 and L17 (see group I in Table 1) was also observed following infection by the mutant phages λ spc1-I15 and λ spc1-I16. The stimulation of the synthesis of many other r-proteins including S5, which is coded for by the *spc*^s gene (group II in Table 1), was, however, greatly reduced or abolished with λ spc1-I16. The reduction in the stimulation of the synthesis of these proteins was in general smaller with λ spc1-I15, but was still significant compared with the group I proteins. There are some proteins (group III in Table 1), whose synthesis was weakly stimulated by λ spc1 phage, or which were difficult to classify into group I or II with respect to the susceptibility of their synthesis to the insertion mutations (see the legend to Table 1).

It should be noted that the effect of the I15 insertion on the stimulation of the synthesis of group II proteins is not uniform. In particular, the stimulation of the synthesis of L24 was almost completely abolished by the I15 insertion. It is possible that the I15 insertion is within the gene for L24.

We have also done similar experiments with several other insertion mutants of λ spc1. As described below, the size of inserted DNA in λ spc1-I15 is different from that in λ spc1-I16. All other insertion mutants could be classified into two classes with respect to the size of inserted DNA, one about the same as that of I15, and the other about the same as that of I16. It was observed that other insertion mutants of "I16 type", like I16 itself, showed marked effects on the synthesis of the group II proteins, but not on that of the group I proteins. Conversely, the effects observed by mutants of "I15 type" were in general similar to those observed with λ spc1-I15: that is, partial inactivation of many or all of group II protein genes and weak or no inactivation of group I protein genes.

These data indicate that there are at least two classes of r-protein genes on λ spc1 whose expression is controlled separately. The insertion mutations studied here seem to appreciably affect one class (group II) but not the other(s) (group I). The weak inactivation found for group I proteins and the difference between the two experiments reported in Table 1 may be a result of slight differences in the multiplicity of infection (see legend to Table 1).

It should be noted that we have proved only that the structural genes for S4, S5, S8, S11, S13 and S14 are carried by λ spc1 since these proteins are made in our DNA-dependent *in vitro* protein synthesising system using λ spc1 DNA as template². It is highly likely, however, that the structural genes for most of the other proteins in groups I and II are also carried by

Table 1 Stimulation of the synthesis of individual r-proteins by λ spc1, λ spc1-I15 (I15) and λ spc1-I16 (I16)

Proteins	$^3\text{H}/^{14}\text{C}$ ratio				% Decrease in stimulation			
	λ S7	λ spc1	I15	I16	I15	I16	I15	I16
Group I								
S4	0.03	6.31	4.78	3.99	24	(15)*	37	(5)*
S11 + S9†	0.08	1.24	1.04	0.84	14	(15)	34	(20)
S13	0.12	7.20	5.31	4.53	27	(20)	38	(18)
L17	0.06	11.68	8.25	7.97	29	(16)	32	(5)
Group II								
S5	0.05	3.60	1.80	0.52	51	(49)	87	(66)
S8	0.04	1.68	0.63	0.11	64	(64)	96	(74)
S14	0.13	3.08	1.32	0.40	60	(50)	91	(75)
L5	0.04	2.50	0.94	0.14	63	(68)	96	(79)
L6	0.03	3.03	1.25	0.73	59	(57)	77	(67)
L14	0.12	5.88	2.83	0.54	53	(44)	93	(79)
L15	0.16	7.97	4.58	0.61	43	(44)	94	(72)
L16	0.05	1.22	0.83	0.37	33	(0)	73	(45)
L18	0.10	4.39	1.72	0.56	62	(50)	89	(65)
L19	0.07	1.29	0.92	0.46	30	(48)	68	(70)
L24	0.13	7.49	0.38	0.31	97	(96)	97	(97)
L30	0.24	6.96	2.59	0.51	65	(52)	96	(65)
Group III								
S3	0.02	0.25	0.13	0.38	52	(41)	(-57)	(44)
S7	0.02	0.36	0.23	0.12	38	(55)	71	(67)
L11	0.05	1.06	0.45	0.16	60	(59)	89	(72)
L13	0.08	0.54	0.37	0.13	37	(41)	89	(69)
L22	0.04	1.05	0.69	0.61	35	(17)	43	(34)
L23	0.06	1.36	1.08	0.73	21	(13)	48	(9)

E. coli cells prelabelled with ^{14}C -leucine were irradiated with ultraviolet light and infected with phages as previously described² except that the ultraviolet-sensitive bacterial strain was a λ papa lysogen of strain 159 (ref. 12). The ^3H -leucine was added 30 min after phage infection and the incorporation was stopped after 10 min. The cells were lysed and proteins were extracted and separated by two-dimensional gel electrophoresis¹³. The $^3\text{H}/^{14}\text{C}$ ratio of each r-protein spot, except S1 and L31, was determined. Details of the procedures were described previously^{2,10,11}. The data are shown only for the proteins whose synthesis was previously found to be stimulated by λ spc1. Synthesis of other proteins was not significantly stimulated by λ spc1 compared with the control phage λ c1857S7 (λ S7). The proteins are classified into groups I and II as described in the text. Group III includes proteins whose synthesis was only weakly stimulated (S3, S7, L13) or whose classification with respect to groups I and II is not clear (L22, L23). The pattern observed with L22 and L23 appears similar to that observed for group I proteins. In the case of L11, the apparent stimulation of its synthesis may be due to contamination of the L11 spot by S5. Concerning a weak reduction by the I16 insertion in the stimulation of the synthesis of group I proteins, we note that stimulation of the synthesis of r-proteins by the transducing phages depends on the multiplicity of infection. In the present experiments, the same 'multiplicity' was used, as judged by the absorbance values of the various phage preparations at 260 nm. This corresponds to multiplicity 5 of infectious λ S7. Thus, the possible presence of some inactive phage particles in the λ spc1-I16 preparation may explain the weak reduction observed in the stimulation of the synthesis of the group I proteins. The slight difference between the two experiments may also be explained on the same basis.

*Similar experiments were done except that the bacterial strain used as a host was a non-lysogenic and ultraviolet-sensitive strain (NO1261)². The values obtained are shown in parentheses.

†Proteins S9 and S11 co-electrophorese in the two-dimensional gel. But, S11 can be made *in vitro* using λ spc1 DNA as template but not S9 (ref. 2). Thus, the stimulation of the incorporation of ^3H -leucine into this spot is probably a result of the synthesis of S11.

λ spc1. Most of these proteins are clearly separated from other proteins in the two-dimensional electrophoresis system used to determine synthesis of r-proteins in the irradiated cells and their synthesis seems to be strongly stimulated by λ spc1 infection. Thus this stimulation probably reflects the presence of the structural gene on λ spc1.

Physical mapping of insertion mutations

The structure of λ spc1, as well as other transducing phages that we have isolated, has been studied by the use of DNA restriction endonucleases (such as EcoRI) and by electron microscopic analysis of various heteroduplexes (Fiandt, Szybalski, Blattner, Lindahl, Jaskunas and Nomura, in preparation). Figure 1 shows the position of various bacterial genes on these transducing phages and the position of the EcoRI sensitive sites on the λ spc1 genome.

Both λ spc1-I15 and λ spc1-I16 had increased phage particle densities (1.489 and 1.491 g ml⁻¹, respectively) relative to the parent λ spc1 (1.486 g ml⁻¹). These increased densities originally suggested that the inactivation of the *spc*⁺ gene on these phages was the result of an insertion. Furthermore, EcoRI digestion of the DNAs from both λ spc1-I15 and λ spc1-I16 showed a fragment pattern identical to that from λ spc1 except that the 17% fragment from λ spc1 was replaced by larger fragments in the mutants. These results indicate that the insertions are

in the original 17% fragment which contains the junction of bacterial and λ DNA ('*coli*- λ junction').

The precise position of the insertions as well as the size of the inserted DNAs was determined by heteroduplex analysis. Examples are shown in Fig. 2. In these experiments, the heteroduplexes formed between the mutant (or parent) DNAs and the DNA from λ fus2 were examined. λ fus2 is a transducing phage carrying the genes *aroE*, *trkA*, *spcA*, *strA*, and *fus*, as well as approximately 28 other r-protein genes (our unpublished experiments, cited in ref. 2). The DNA in λ spc1 up to the *coli*- λ junction is homologous to DNA in λ fus2 (see Fig. 1). The positions of the insertions obtained from the heteroduplex experiments are indicated in Fig. 1. The I16 insertion was found 270 ± 30 base pairs left of the *coli*- λ junction and the I15 insertion was found 850 ± 40 base pairs left of the junction. Thus, both insertions are in the bacterial DNA. Since the DNA between either insertion and the *coli*- λ junction can code for only one to three r-proteins, we conclude that most or all of the inactivated genes are to the left of the insertions. Independent measurements of the distance between the two insertion sites in the heteroduplex from λ spc1-I15 and λ spc1-I16 (Fig. 2d) gave the value 610 ± 60 base pairs, which agrees with the calculated value, 580 ± 50.

The sizes of the inserted DNAs were measured and found to be 680 ± 70 base pairs in λ spc1-I15 and 1,160 ± 110 base pairs in λ spc1-I16. Thus the sizes of these two inserted DNAs

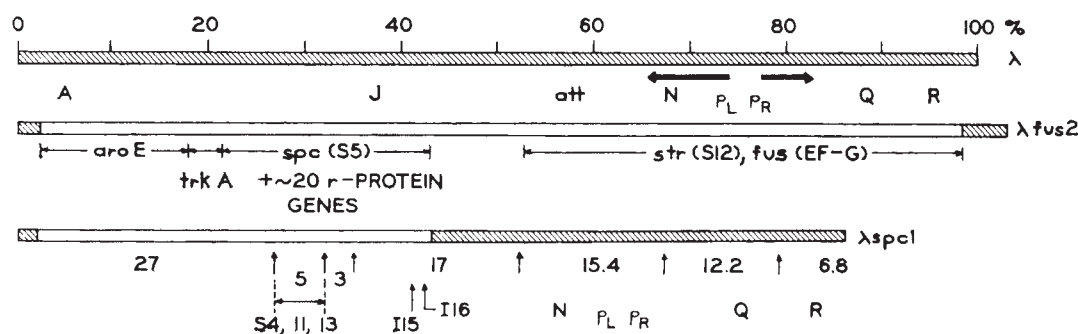


Fig. 1 The structure of λ , $\lambda fus2$ and $\lambda spc1$. DNA from the λ genome is hatched. Arrows under the $\lambda spc1$ chromosome show the *EcoRI* sensitive sites which produce seven DNA fragments. The size of these fragments is indicated in % λ units. The *str* gene is present in $\lambda fus2$, but not in $\lambda spc1$. The structure of $\lambda fus2$ and $\lambda spc1$ is based on the experiments by M. Fiant, W. Szybalski, F. Blattner, L. Lindahl, R. Jaskunas and M. Nomura (in preparation). Two thick arrows under the λ chromosome indicate the leftwards transcription from the P_L promoter and rightwards transcription from the P_R promoter, respectively. The location of genes for S4, S11 and S13 as well as that of the insertion mutations, I15 and I16, is indicated.

are similar to the sizes of the well-studied insertion DNAs, IS1 (750 to 870 base pairs) and IS2 (1,170 to 1,420 base pairs), respectively^{4,5}. The slight difference between our value for I15 and the published values for IS1 is probably not significant.

Direction of r-protein gene transcription at the *str-spc* region

$\lambda spc1$ phage carries about 20 r-protein genes. The data described above show that the insertion of small DNA fragments depresses the expression of many r-protein genes located left of the site of the insertion (compare Fig. 1). The simplest interpretation of the results is that these inactivated genes belong to one transcriptional unit and that the direction of transcription is from right to left in Fig. 1.

Previous experiments suggested that the direction of the transcription of r-protein genes in this region is from *spc* to *str* (ref. 3 and other experiments cited in ref. 1). Since that is opposite to the above expectation, we have reinvestigated the direction of the transcription by determining which strands of $\lambda spc1$ and $\lambda fus2$ hybridise with the r-protein messages for this region.

First, we separated the strands of DNA from $\lambda trkA$, $\lambda spc1$ and $\lambda fus2$ and determined which strands would hybridise 'leftwards' transcripts and which would hybridise 'rightwards' transcripts. $\lambda trkA$ is a control phage which has only the *aroE-trkA* end of $\lambda spc1$ and $\lambda fus2$. It does not seem to code

for any r-proteins². For $\lambda trkA$ and $\lambda spc1$, identification of the polarity of the separated strands was done with purified λ -l and λ -r transcripts. We found that the 'heavy' strand of $\lambda trkA$ hybridised with the λ -r transcript while the 'light' strand of $\lambda spc1$ hybridised with this transcript (Table 2). This method could not be used to determine the polarity of the $\lambda fus2$ strands since they did not hybridise a significant fraction of the standard λ transcripts, presumably because this phage contains only a small fraction (7%) of the λ genome. We therefore used the *in vitro* transcript from the 5% *EcoRI* fragment of $\lambda spc1$ (compare Fig. 1). This transcript ('5% transcript') contained mainly 'leftwards' transcript since it hybridised primarily with the $\lambda spc1$ -l strand (Table 2). $\lambda fus2$ also contains the 5% *EcoRI* fragment and we found that its 'heavy' strand hybridised with the 5% transcript establishing it as the $\lambda fus2$ -l strand (Table 2).

It was previously shown that the *in vivo* synthesised RNA which hybridises to $\lambda spc1$ DNA is almost exclusively r-protein mRNA and its major fraction is homologous to the bacterial DNA where the genes affected by I15 and I16 are located (P. Dennis and M. Nomura, unpublished). Data presented in Fig. 3 show that *in vivo* synthesised mRNA hybridised with the l-strands of $\lambda spc1$ and $\lambda fus2$ but not with their r-strands. We conclude that *in vivo* transcription of the r-protein genes in the *str-spc* region seems to be exclusively leftwards, that is, in the direction of *str* to *spc*.

Table 2 Hybridisation of *in vitro* transcribed RNA to separated DNA strands of transducing phages

Experiment No.	RNA Type	Input c.p.m.	λ		$\lambda trkA$		c.p.m. Hybridised to*		$\lambda fus2$	
			H	L	H	L	H	L	H	L
1	λ -r	655	561	23	461	13	44	238	11	49
2	λ -l	560	33	406	27	411	246	49	18	9
3	"5%"	38,983	—	—	104	68	17,631	1,608	10,001	252
4	" $\lambda spc1$ "	27,707	3,439	1,563	3,103	2,355	9,759	3,875	5,510	769

Binding to filters in the absence of DNA [(1) 28 c.p.m.; (2) 22 c.p.m.; (3) 26 c.p.m.; (4) 30 c.p.m.] has been subtracted. Preparation of transcripts from the λ -strands: ^3H -labelled transcripts from λ DNA were made in a system with the following composition: 10 mM Tris-HCl (pH 7.9), 10 mM MgCl₂, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.5 mM K-phosphate, 60 mM KCl, 6% glycerol, 0.6 mM ^3H -UTP (1.3 Ci mmol⁻¹), 0.5 mM CTP, 0.5 mM ATP, 0.5 mM GTP, 11 $\mu\text{g ml}^{-1}$ λ DNA and 240 $\mu\text{g ml}^{-1}$ protein of an RNA polymerase preparation, which is approximately 50% pure¹⁴. The mixture was incubated at 37 °C for 45 min. The reaction was terminated by chilling to 0 °C, 200 μg tRNA was added as carrier and nucleic acids purified by the phenol method. From this preparation 'leftwards' (λ -l) and 'rightwards' (λ -r) transcripts were purified by preparative hybridisation to λ -L and λ -H strands essentially as described by Bøvre *et al.*¹⁵. '5% transcript' was prepared in a DNA-dependent protein synthesising system as described previously¹¹, except that RNA was labelled (with 8.5 Ci mmol⁻¹ ^3H -UTP) rather than protein. The template used was a 5% fragment of $\lambda spc1$ DNA prepared by *EcoRI* digestion (see Fig. 1). Its molecular weight is about 1.4×10^6 and it codes for the r-proteins S4, S11 and S13 (Lindahl, Zengel and Nomura, in preparation). $\lambda spc1$ transcript was prepared using $\lambda spc1$ DNA as a template according to the method used for the λ transcripts (see above). Separation of strands was performed by annealing with poly(U,G) and isopycnic CsCl centrifugation as previously described¹³⁻¹⁸. Liquid hybridisation was performed in 2 $\times$ SSC using a large excess of DNA. Samples were incubated at 67 °C for 4–6 h and collected on Millipore HAWP nitrocellulose filters. Each filter was washed with 50 ml 2 $\times$ SSC. The filters were then incubated in 20 μg pancreatic and 2.5 units T1 ribonucleases ml⁻¹ in 2 $\times$ SSC (1.5 ml per filter) for 1 h at 37 °C. Finally the filters were washed twice in 2 $\times$ SSC (10 ml per filter), dried, and the radioactivity bound to each filter was measured. SSC contains 0.15 M NaCl, 0.015 M Na citrate, pH 7.2.

*DNA strand H and L designate separated strands which were located at heavier and lighter density positions, respectively, in the isopycnic CsCl centrifugation after annealing with poly(U,G).

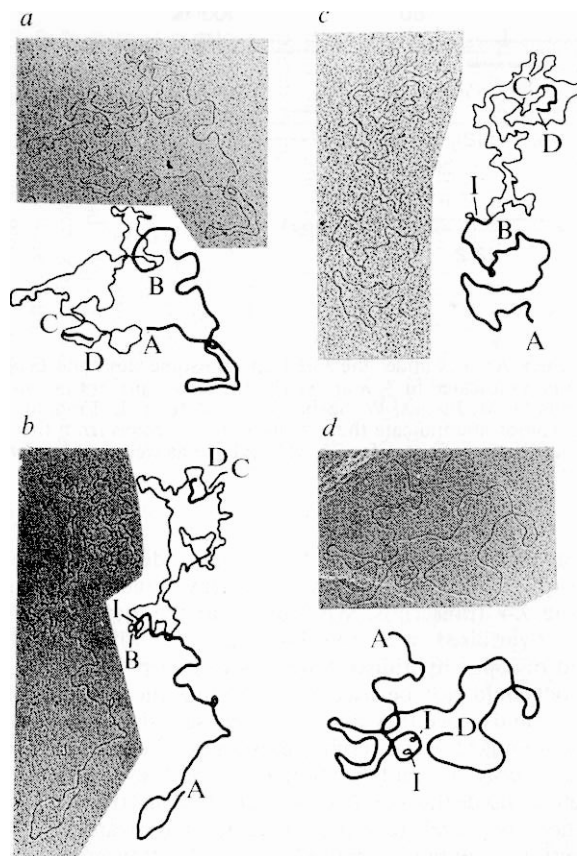


Fig. 2 Electron micrographs of heteroduplex DNA molecules. *a*, λ spc1 and λ fus2 DNA; *b*, λ spc1-I16 and λ fus2 DNA; *c*, λ spc1-I15 and λ fus2 DNA; *d*, λ spc1-I16 and λ spc1-I15 DNA. Both phage PM2 DNA (9,500 base pairs; refs 19 and 20 and W. Szybalski, personal communication) and Φ X174 DNA (5,250 base pairs; ref. 20) were added as references for length measurements. In *a* and *b*, one PM2 DNA molecule overlaps on the heteroduplex molecules. The tracings of each heteroduplex are shown together with the electron micrographs. Heavy lines are duplex regions, and light lines are single-strand regions. Several key positions are indicated as follows: A, the left end of the heteroduplex molecule (compare Fig. 1); B, the position of the right *coli*- λ junction in λ spc1; C, the position of the right *coli*- λ junction in λ fus2; D, the right end of the heteroduplex molecule; I, the position of insertion (I15 or I16). The method for preparation of heteroduplexes and for electron microscopy was according to ref. 16. The lengths of double strand DNA regions were measured using PM2 DNA as a reference. The lengths of single strand DNA regions were measured using Φ X174 DNA as a reference, but in some experiments, PM2 DNA was used as a reference. No significant difference was observed between the values obtained with the two methods.

Both λ spc1 DNA and its 5% *Eco*RI fragment code for r-proteins in our DNA-dependent protein synthesising system. We found that the *in vitro* transcripts from these DNAs also hybridised predominantly to the *l*-strand of λ spc1 DNA (Table 2). Since the 5% fragment seems to contain only r-protein genes (L. L., J. Zengel and M. N., unpublished), we conclude that the *in vitro* transcripts from this fragment contain predominantly r-protein messengers. *In vitro* transcripts of λ spc1 seem to contain transcripts from the λ genes in addition to the transcripts from the bacterial genes, as can be seen from hybridisation to the separated λ DNA. To test the transcripts from the bacterial DNA, we performed hybridisation experiments using separated λ fus2 DNA strands which contain only a small fraction of the λ genome. The results showed that the *in vitro* transcripts for λ spc1 hybridised mainly with the λ fus2-*l* strand (Table 2, experiment 4). Thus, these results are consistent with the finding that *in vivo* synthesised r-protein mRNA for the genes on λ spc1 are transcribed leftwards and

indicate that in general the correct strand is being transcribed under our conditions for *in vitro* transcription.

Multiple transcriptional units

We have shown that insertion mutations, I15 and I16, greatly reduce the expression of many r-protein genes present on the λ spc1 genome. A few of the r-protein genes on this phage, however, are apparently not affected to any large extent by these insertions. Among them, the genes for S4, S11, and S13 have been shown to be closely linked to each other and are mapped on the 5% *Eco*RI fragment (Fig. 1 and L. L., J. Zengel and M. N., unpublished). Most of the genes inactivated by the I15 or I16 insertions (genes for the group II proteins in Table 1) have been mapped between the gene cluster for S4, S11 and S13 and the *coli*- λ junction using several deletion mutants of transducing phages (our unpublished experiments). Because the direction of the transcription is from right to left, as demonstrated in our present work, the inactivated genes are distal to the insertion. Although the mechanism of this inactivation of the distal genes by these insertions is not known, the size of the inserted DNA as well as their "polar effect" resemble those of the IS1 and IS2 insertions studied in other systems⁴⁻⁶. It is very likely that the r-protein genes affected by the I16 (or I15) insertion belong to a single transcriptional unit, and those not affected (S4, S11 and S13, and others) belong to another one or more transcriptional units. Our present data cannot, however, exclude the possibility that there

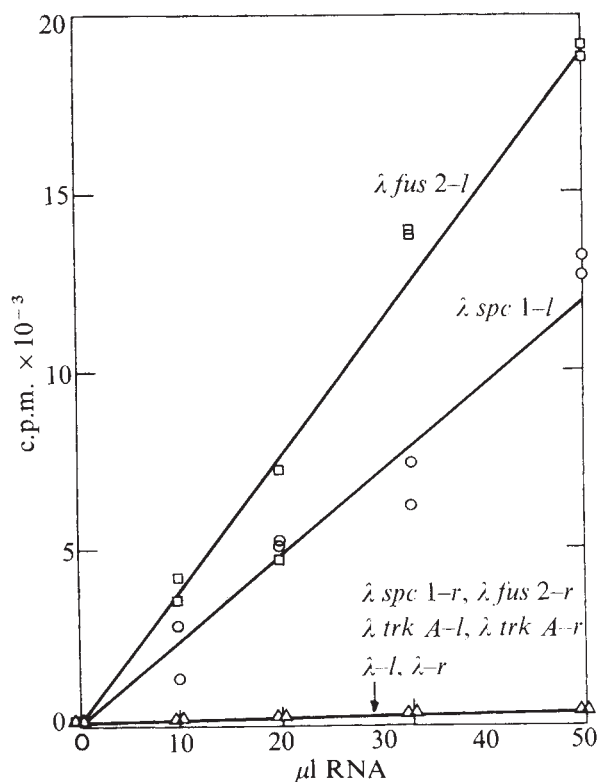


Fig. 3 Hybridisation of *in vivo* RNA to separated strands of λ , λ trkA, λ spc1, and λ fus2. Various amounts of RNA prepared from pulse labelled *E. coli* K12 (see below) were hybridised to DNA as indicated. All strands were used in an excess over RNA. Triangles show the average of the bindings to the indicated strands. Binding to individual strands differs slightly, but not enough to be shown on this scale. Preparation of RNA: *E. coli* K12 PR13 (ref. 17) was grown exponentially in glucose-amino acid medium at 37 °C¹¹. At approximately 10⁸ cells ml⁻¹ the culture was labelled with 0.5 μ g ³H-uracil (25 Ci mmol⁻¹) ml⁻¹ for 4 min. The pulse was terminated by pouring the culture on crushed ice. RNA was prepared from the cells essentially as described before¹¹. The RNA preparation had 1.2 × 10⁷ c.p.m. ml⁻¹. Conditions for hybridisation are described in the legend to Table 2.

is a "secondary" promoter between the genes affected and those not affected and that all the r-protein genes carried by λ spc1 are cotranscribed in "normal" conditions. The activity of such a secondary promoter could be enhanced by an upstream polar mutation. The present data also cannot exclude the possibility that the genes inactivated by a single insertion belong to more than one transcriptional unit and the inactivation involves a more complicated mechanism. Thus, further studies are required to elucidate the mechanism of the apparent polar effect observed in the present system.

It should also be noted here that while the polar effects resulting from IS1 insertions are very strong in other systems, those resulting from the I15 insertion in our system are only partial. The question whether the IS1 DNA is identical to the DNA inserted in λ spc1-I15 must await further experiments.

The genes on λ spc1 that code for the group II proteins (Table 1) can function *in vivo* in the presence of the λ repressor (experiments in Table 1 and unpublished data) and so the transcription probably starts from a bacterial promoter, rather than a λ promoter. Such considerations would suggest the presence of a bacterial promoter close to the *coli*- λ junction in λ spc1. No experimental proof has been obtained to support this conjecture, however.

Previous experiments using phage Mu-1 suggested that the r-protein genes in the *str*-*spc* region are transcribed in the direction of *spc* to *str* (ref. 3 and other experiments cited in ref. 1). Our present results demonstrate that the opposite direction is correct. We cannot offer any simple explanation for the results of the Mu insertion experiments. In the previous experiments, the genes inactivated by the Mu phage insertion were not recovered and thus the possibility that these genes were deleted rather than inactivated by a polar effect was not eliminated.

Previous physiological studies on the synthesis of r-proteins in *E. coli* suggested the presence of several transcriptional units for r-protein genes⁷. It is now clear that the organisation of r-protein genes is complex. Many r-protein genes (about 30) are clustered at the *str*-*spc* region. Our results strongly suggest that they are organised into at least two transcriptional units. Furthermore, we have previously shown that at least one r-protein gene, the structural gene for S18, is mapped outside

the *str*-*spc* region^{8,9}. We, and others, have also observed that approximately five other r-protein genes are clustered near *rif* which is far away from the *str*-*spc* region (S. R. J., L. L. and M. N., unpublished and J. Friesen, personal communication). Thus, coordinated synthesis of all the r-proteins^{10,11} requires a mechanism which coordinates the expression of several r-protein gene transcriptional units.

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Simplest statistical geometric model of the simplest version of the multicomponent random packing problem

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A simple, statistical, geometric model of multicomponent random disk packing, which ignores the gaps that occur in real packings, is compared with experimental packings. The model gives a good qualitative description of the changes that occur in packing structures as the proportions of the disks in a mixture changes, and considers the different types of contact that occur between disks. The average coordination for disk packings is constant for all mixture proportions.

The sphere packing problem, common to many fields of science and technology, arises in studies of systems involving bubbles, drops, grains, fibres, molecules, and so on¹.

The packing of monosize components is, however, considered

most often and then from the point of view of overall or average properties (such as porosity). That is in spite of the fact that, in practice, mixtures of different sized components are nearly always encountered; in cases such as these the distribution effects (for example pore-size distribution) are very important. In such cases, however, the calculation of the fine structure of the packing is impossible and even measurement is difficult. We therefore present here an alternative approach.

The two-dimensional packing of a mixture of two disk sizes, probably the simplest possible expression of the sphere packing problem, can be considered by using a model designed for multicomponent sphere packing^{2,3} but reduced here from three to two dimensions.

The basic hypothesis is that in a random arrangement of disks each disk touches its neighbours. This allows the space to be divided up into triangles formed by lines joining the

centres of contiguous disks (Fig. 1). The hypothesis is, therefore, that gaps, such as those shown by the dotted lines in Fig. 1, do not exist.

This abstraction of the problem allows the effects of size distribution to be considered apart from the less easily defined effects of the perturbation of the structure caused by inevitable,

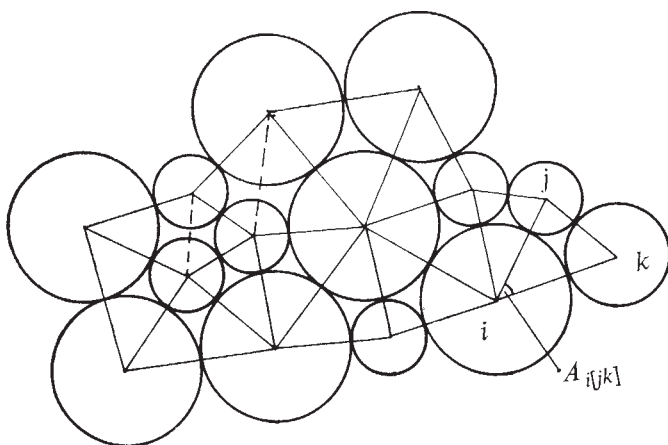


Fig. 1 Binary disk packing.

irregular gaps. That is, elementary subunits in the packing are calculated but their subsequent assembly to form the packing structure is ignored. The model is limited to the case in which there is interaction between the sizes; effectively to cases in which the diameter ratio is less than 1:6.46. Above that value it is possible to fit a small disk in the space between three large contiguous disks without touching them.

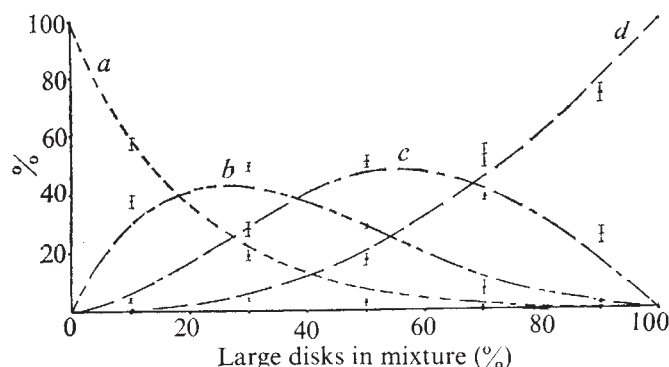
Mathematical statement of the model

In the case of the binary packing of disks of sizes 1 and 2 the space is divided into four types of triangular subunits, each formed by groups of three disks—111, 112, 122, 222. More generally, in an N component disk packing there will be

$$N(N+1)(N+2)/3! \quad (1)$$

different types of triangle. Once the frequency distribution of these different triangles is known, then all of the properties of the idealised packing can be deduced: the porosity, the pore size distribution, the mean coordination number for each disk size, and so on.

Fig. 2 Triangle frequency distribution. Type 111: $\times$, experimental distribution; a , model distribution. Type 112: $\bullet$, experimental distribution; b , model distribution. Type 122: $+$, experimental distribution; c , model distribution. Type 222: $\circ$, experimental distribution; d , model distribution.



The triangle frequency distribution must be calculated from the relative numbers and sizes of the disks. Two effects come into play: size and number. For example, if there are more disks of diameter 1, then triangles including disks of diameter 1 will be favoured. On the other hand, disks of diameter 2, being bigger, will have more other disks touching them and will form more triangles, therefore, triangles including disks of diameter 2 will be favoured. The probability of a disk taking part in a triangle will be related to Z_i :

$$Z_i = n_i C_i$$

where n_i is the fractional number frequency of disks of size i in the packing, and C_i is the average number of contacts on disks of size i in the packing.

If triangle systems are obtained by random combinations of groups of three disks, then the frequency distribution of triangles in a binary mixture will be given by the respective terms of the expansion of the expression

$$(Z_1 + Z_2)^3 \quad (2)$$

or, in a form suitable for programming,

$$(3!/a!b!)(Z_1)^a(Z_2)^b \quad (3)$$

where a or $b = 0, 1, 2$ or 3 and $a+b = 3$. That is, the relative frequency of triangles of type 112 is given by

$$(3!/2!1!)(Z_1)^2(Z_2)^1 \quad (3)$$

The mean coordinate number for a given disk size is the reciprocal of the mean angle subtended at that type of disk by other disks in the packing, expressed in fractions of a circle. This is not known *a priori* and must be calculated by trial and error. Equations (1)–(3) show that:

$$C_j = (Z_i + Z_j + Z_k + \dots)^2 / A' \quad (4)$$

where

$$A' = (A_{j(ij)} Z_i^2 + A_{j(jj)} Z_j^2 + A_{j(kk)} Z_k^2 + \dots) + 2(A_{j(ij)} Z_i Z_j + A_{j(ik)} Z_i Z_k + A_{j(jk)} Z_j Z_k + \dots)$$

$A_{i(jk)}$ (Fig. 1) is the angle subtended at the centre of disk i by the lines joining its centre with the centres of two contiguous disks, j and k . For diameter ratios up to the maximum possible considered here (1:6.46), to within about 1%:

$$(A_{j(ij)})^{\frac{1}{2}} \times (A_{j(jj)})^{\frac{1}{2}} \approx A_{j(ik)} \quad (5)$$

This allows equation (4) to be simplified to

$$C_j = \left[\sum_{i=1}^{i=N} Z_i \sum_{i=1}^{i=N} K_{ji} Z_i \right]^2 \quad (6)$$

where $K_{ji} = (1/C_{ji})^{\frac{1}{2}}$ and C_{ji} is the number of contacts on a disk type j surrounded completely by disks type i . Equations like (4) or (6) can be solved rapidly and efficiently using the Newton-Raphson technique.

In all calculations made with ternary as well as binary mixtures, with diameter ratios up to the maximum allowed by the model:

$$\sum_{i=1}^{i=N} n_i C_i = \text{constant} \quad (7)$$

For disks, this constant is 6.0.

No formal proof of this has been obtained but the reproducibility of the value 6.0 suggests something more than a

purely fortuitous relationship of the type seen in equation (5). This is perhaps connected with the proven fact that the average Voronoi polygon of any array of points in two dimensions has exactly six sides⁴.

Experimental binary disk packings

Experiments have been performed with large disks (20 mm and 40 mm diameter) to be able to distinguish between real contacts and close contacts, based on differences of about 1% of a diameter. Disks were made from steel washers machined to be perfectly circular with a tolerance on the diameter of ± 0.2 mm. To form a two-dimensional packing they were introduced into a

in Fig. 1. The numbers of the different types of 'false' triangle were then counted (Table 1 and Figs 2 and 3).

Figure 2 is the sum of the distributions of the 'true' and the 'false' triangles, as it was found that these two distributions did not differ greatly from each other.

The model

From the results in Figs 2 and 3 and the ratios C_4/C_2 (Table 1), it can be seen that the model gives a good qualitative picture of the variations in packing characteristics with mixture proportions. Table 1 shows also that the total number of contacts on a given number of disks is approximately constant,

Table 1 Model and experimental results

Disk diameter	%	Number of triangles per experiment			Model C_4/C_2	Coordination numbers				
		True	False	C		Σ_{nc}	C	σ	Experiment C_4/C_2	Σ_{nc}
2	90	274	396	5.71			4.39	0.74		
4	10			8.64	1.51	6.00	6.89	0.77	1.39	4.64
2	70	197	188	5.25			4.18	0.64		
4	30			7.74	1.47	6.00	6.49	0.88	1.56	4.87
2	50	148	196	4.91			3.82	0.63		
4	50			7.09	1.35	6.00	5.68	0.78	1.49	4.75
2	30	96	212	4.63			3.56	0.51		
4	70			6.59	1.42	6.00	5.10	0.74	1.44	4.64
2	10	82	121	4.41			3.15	0.64		
4	90			6.18	1.40	6.00	4.88	0.75	1.54	4.71

360 by 600 mm Perspex frame one by one in groups of ten, with the required mixture proportions. The packing was not shaken, so as to avoid segregation effects which can be important in mixtures. Each packing was repeated at least twice and represents a counting of around 500 disks, depending on size.

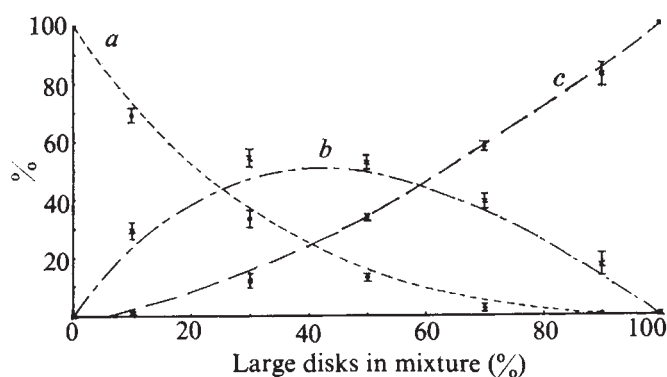


Fig. 3 Types of contact. Type 11: ●, experimental; a, model. Type 12: ×, experimental; b, model. Type 22: ■, experimental; c, model.

Three different mixtures were used. A tracing of the assembly was made and the various countings were made on diazo prints of this tracing. In all cases, disks at the edges to a depth of about two disks were excluded to minimise edge effects.

The centres of contiguous disks were joined and several different counts were made: the numbers of the different types of 'true' triangle; the numbers of contacts on each disk size; the numbers of the different types of contact—that is, between similar disks—1,1 and 2,2—and between dissimilar disks—1,2. The remaining polygons were divided into triangles by joining the closest non-touching disks, as by the dotted lines

as is predicted by the model.

The model describes high density packing with a maximum number of contacts between disks. Not all of these contacts are made, however; it seems that the selection of the contacts not to be made is done randomly and that the gaps do not tend to be associated preferentially with either of the two disk sizes. The influence of the gaps is found only in the absolute values: for example, the total number of contacts per unit sample of disks is 4.72 and not 6. The fact that this reduction is uniform for all mixtures could be used to make the model quantitatively predictive.

It may be concluded that the triangle frequency distribution and the distribution of contact types are not much disturbed by the presence of the gaps. This indicates that, contrary to the influence of the walls, the gaps do not have any ordering influence and that, as the model assumes, the disks touch each other on the basis of random encounters governed only by their relative size and number.

The results also provide some insight to another feature of binary packings, which has been noticed before. Since each type of triangular unit has its own characteristic porosity the variation of the overall porosity with mixture proportions is given by a weighted sum of the four distribution curves in Fig. 2. This provides an explanation for the occurrence of the well known V-shaped, porosity/mixture proportion curves for binary packings. This effect is much more noticeable for sphere packings or disk packings with a wider diameter ratio.

Several points emerge from this study: packings are formed by random encounters between disks; the model predicts the correct distribution of contact types and triangular subunit types; the total number of contacts in a unit sample of disks is constant for all mixture proportions; the gaps in the packing occur in a random fashion.

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letters to nature

Discovery of a corona around an early-type star and the problem of mass loss

THE star τ Sco, HD149438, spectral type B0 V, was observed spectroscopically by the Princeton telescope-spectrometer on the Copernicus satellite during June and July 1973. This star is well known¹ as being one of the few early type stars with a small projected rotational velocity, $v \sin i = 20 \text{ km s}^{-1}$, thus showing narrow spectral lines with a minimum of overlap or blending. So the spectrum of the star was scanned with a resolution of $0.05 \text{ \AA}$ over the entire wavelength range from $950 \text{ \AA}$ to $1,560 \text{ \AA}$ to obtain a prototype ultraviolet spectrum for an early-B star. This spectrum is in preparation by Rogerson and Upson.

Apart from the numerous spectral features with a full width at half maximum (FWHM) of about 20 km s^{-1} which originate in the photosphere of the star, and the interstellar absorption lines, the spectrum also shows a few very wide absorption lines. These lines are identified as belonging to the spectra of O VI at $1,032$ and $1,038 \text{ \AA}$, NV at $1,239$ and $1,243 \text{ \AA}$ and Si IV at $1,394$ and $1,403 \text{ \AA}$. The lines are highly asymmetric with the largest depression close to the laboratory wavelengths and an enhanced short-wavelength wing, which extends to a Doppler velocity of about $-1,000 \text{ km s}^{-1}$. This suggests that the lines are formed in the expanding outer layers of the atmosphere.

The strong presence of O^{++} and N^{++} ions in the outer layers of the star is surprising. From the visual and ultraviolet spectrum the temperature of the stellar photosphere is determined to be about $30,000 \text{ K}$ and the stellar atmosphere models predict that the temperature in the layers above the photosphere is less. To produce a significant fraction of the observed highly ionised atoms, the temperature in the outer layers must be² of the order of 1×10^5 – $5 \times 10^5 \text{ K}$. (The production of O^{++} and N^{++} by radiative ionisations can be neglected since the required stellar radiation in the far ultraviolet is very strongly absorbed in the photosphere by neutral hydrogen atoms and ionised helium atoms.) Therefore, the temperature in the atmosphere of τ Sco must decrease with height in the photosphere, go through a minimum in higher layers and then increase again to at least a few hundred thousand K.

A similar temperature structure is known in the solar atmosphere, where the minimum is about $4,200 \text{ K}$ and from there on the temperature increases outward throughout the chromosphere until it reaches a more or less constant value of about $2 \times 10^6 \text{ K}$ in the corona. The large temperature is due to the dissipation of mechanical energy in the form of acoustic waves in the lower chromosphere and possibly by Alfvén waves in the corona³. The mechanical energy in the Sun is supposed to originate in the unstable hydrogen convection zone below the photosphere.

Main sequence stars with effective temperatures greater than about $8,300 \text{ K}$ are not expected to have well developed hydrogen convection zones nor to generate a large mechanical energy flux which could produce a stellar corona⁴.

A possible mechanism for generating a mechanical energy flux in hot stars has been proposed by Hearn^{5,6}. He has pointed out that in the presence of a strong radiation field,

such as in early type stars, density waves can grow and propagate outwards due to the absorption of radiation. This theory predicts a mechanical energy flux of the order of $10^7 \text{ erg cm}^{-2} \text{ s}^{-1}$ for a main sequence B0 star, which in analogy to the solar chromosphere, seems to be sufficient to heat the outer layers to a few hundred thousand K.

The presence of a high temperature region (called a corona, in analogy to the solar case) around early type stars may provide the clue to the understanding of mass loss from hot stars. The observed expansion velocities are generally accepted to be caused by radiation pressure acting primarily on the resonance lines of abundant ions⁷. An important limitation to the efficiency of this mechanism is the fact that the line transitions which are most suitable for absorbing momentum from the radiative flux in the outer atmospheric layers are also extremely effective in the photospheric layers in the reduction of the needed flux. This problem can be illustrated by the very small rates of mass loss predicted by Lucy and Solomon for stars with effective temperatures slightly greater than $30,000 \text{ K}$, at type B0, where the photospheric C IV lines are very strong.

This difficulty is overcome if the outer atmosphere is hotter than the photosphere, because the abundant ions in the outer atmosphere are different from the photospheric constituents. The presence of O VI ions in the corona, having resonance lines at wavelengths near the stellar flux maximum, is especially important in this respect. If 10% of the coronal oxygen atoms are in the form of O^{++} and the oxygen abundance is the same as in the Sun, the outward directed radiative force exerted on the gas due to the O VI lines exceeds the inward directed gravitational attraction by a factor of four in τ Sco. A preliminary estimate indicates a rate of mass loss of about $10^{-8} M_{\odot} \text{ yr}^{-1}$. This is about two orders of magnitude less than the mass loss rate from the Orion OB supergiants⁸.

How unique is τ Sco? In its visible spectrum there is no indication that the star is different from other B0 V stars, except for its small projected rotational velocity. If the star is normal, as we expect, other early-B stars may be surrounded by coronae as well. A preliminary check of the Copernicus files has indicated that traces of O VI lines are detectable on the lower resolution spectra of several other early type stars. Further details will be published elsewhere.

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North-south component of the interplanetary magnetic field and large scale auroral dynamics

THE role of the north-south component B_z of the interplanetary magnetic field on magnetospheric processes, in particular on magnetospheric substorm processes, has caused some controversy¹⁻³. It has been generally thought that the southward turning of the B_z component leads directly to a series of growth phase processes which trigger the expansive phase of substorms. On the other hand, auroral observations made along the meridian chain of stations in Alaska showed that substorms occur frequently along a contracted auroral oval (namely, beyond the poleward field of view at auroral zone stations) even when the B_z component is directed northward ($B_z > 0$)⁴.

Fortunately, both large scale auroral photographs taken from the DMSP satellite (October and December 1972, and January 1973), and the interplanetary magnetic field data during the corresponding periods (from the HEOS satellite) have recently become available. The results are ideal for

frequency of substorms for contracted ovals⁵. Thus, on the basis of the ISIS-2 and DMSP-HEOS satellite data, one can conclude that the occurrence of substorms does not depend on the size of the oval, except when the oval becomes minimum (that is, during prolonged periods of a large positive B_z component).

I now examine theoretical implications of this finding. Let ϕ_D be the production rate of merged (or open) field lines along the dayside X line. This quantity is also believed to be equal to the potential drop along the X line and also to the potential drop across the dawn-dusk meridian in the polar cap^{6,7}. ϕ_N is the production rate of reconnected (or closed) field lines along the nightside X line.

Consider the quantity defined by

$$S = \int_0^\tau (\phi_D - \phi_N) dt$$

where the time $t = 0$ is chosen at the time when the interplanetary magnetic field begins to decrease after having a large northward component for an extended period. Note that the quantity S defined here is equal to $(A_1 - A_0)B_p$, where A_0 and A_1 denote the area of the minimum size oval and of an expanded oval, and B_p denotes the magnetic field intensity

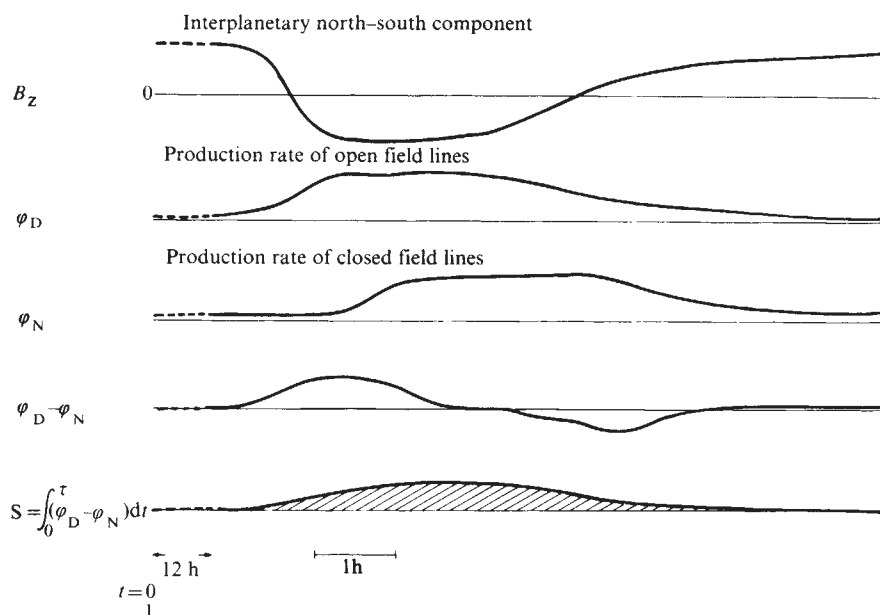


Fig. 1 Hypothetical change of the B_z component and the resulting changes of ϕ_D , ϕ_N , $\phi_D - \phi_N$ and

$$S = \int_0^\tau (\phi_D - \phi_N) dt.$$

settling the controversy; it seems that the occurrence of substorms does not depend on the size of the auroral oval, except for periods when the oval contracts to its minimum size (midnight latitude $\approx 72^\circ$).

Full details of this study will be reported in detail elsewhere. In brief, the occurrence of auroral substorms depends on neither the sign of the B_z component, nor the sign of the dB_z/dt , except for prolonged periods (≥ 6 h) of the large ($\geq 5\gamma$), steady, positive (northward) B_z field. During these exceptional periods, the midnight portion of the oval contracts to invariant latitude of about $72^\circ \sim 73^\circ$ (the highest latitude observed so far for a quiet oval). Thus, this contracted oval can be called the minimum size oval. No substorm features are observed along the minimum size oval; the corresponding state of the magnetosphere may be considered to be the 'ground state'.

What magnetospheric quantities can one infer, on the basis of these findings, as a controlling factor of the occurrence of substorms? Photographs taken from ISIS-2 have already indicated that there is no drastic decrease of the occurrence

in the polar ionosphere. Thus, we may write

$$S = \int_0^\tau (\phi_D - \phi_N) dt = B_p(A_1 - A_0)$$

Figure 1 illustrates schematically time variations of ϕ_D , ϕ_N , $(\phi_D - \phi_N)$ and S when the north-south component of the interplanetary magnetic field B_z varies from a large positive value to a negative value for about two hours and then back to a large positive value again. Note that an extended period of a large positive B_z value is the initial condition.

The immediate response of ϕ_D to the B_z change has been observed⁸; the motion of the cusp or of the mid-day aurora towards the Equator closely follows B_z changes. But it is not yet known accurately how rapidly the midnight portion of the oval responds to the initial B_z change. This depends on how quickly the information on a change of ϕ_D can reach the nightside X line and the production rate ϕ_N begins to

respond to it. The minimum delay time τ_m can be roughly estimated by

$$\tau_m = B_p(A_1 - A_0)/\phi_D \approx 40 \text{ min}$$

where

$$\phi_D = (400 \text{ km s}^{-1}) \times (5\gamma) \times (15R_E)$$

and A_0 and A_1 are assumed to be bounded by the latitude circles of 72° and 65° , respectively.

Because of this delay, the quantity $(\phi_D - \phi_N)$ should increase initially. This quantity is directly proportional to the rate of change of the polar cap area, namely the area bounded by the auroral oval. When $(\phi_D - \phi_N)$ is positive, the oval expands. But ϕ_N begins to increase about 40 min to 1 h after $t = 0$, and eventually a new steady state $\phi_D = \phi_N$ will be reached. Then the auroral oval ceases to expand.

Suppose the B_z then begins to increase after maintaining a large negative value for about two hours. The quantity ϕ_D responds immediately to the B_z change and begins to decrease. But the corresponding ϕ_N variation will again be delayed for about 40 min to 1 h, until the new information on B_z can reach the nightside X line. During this period, the quantity $(\phi_D - \phi_N)$ becomes negative, and thus the oval contracts. If a large B_z value is maintained for a prolonged period, both ϕ_D and ϕ_N reach the same minimum value.

Meanwhile, the quantity S increases until $(\phi_D - \phi_N)$ becomes zero, and then begins to decrease. Eventually, S will become zero after a prolonged period of a large B_z value (that is, when both ϕ_D and ϕ_N reach the same minimum value).

In constructing Fig. 1, I assumed that both ϕ_D and ϕ_N have a finite value even when the B_z component has a large positive value. This is because the magnetotail (a product of the solar wind-magnetosphere dynamo) seems to be a permanent feature. At the lunar distance ($60R_E$), there is no significant difference in the structure of the magnetotail for both low K_p values ($K_p \leq 1+$) and high K_p values ($K_p \geq 2-$)¹⁰. Thus, there must always be a finite amount of open flux ($= B_p A_0$),

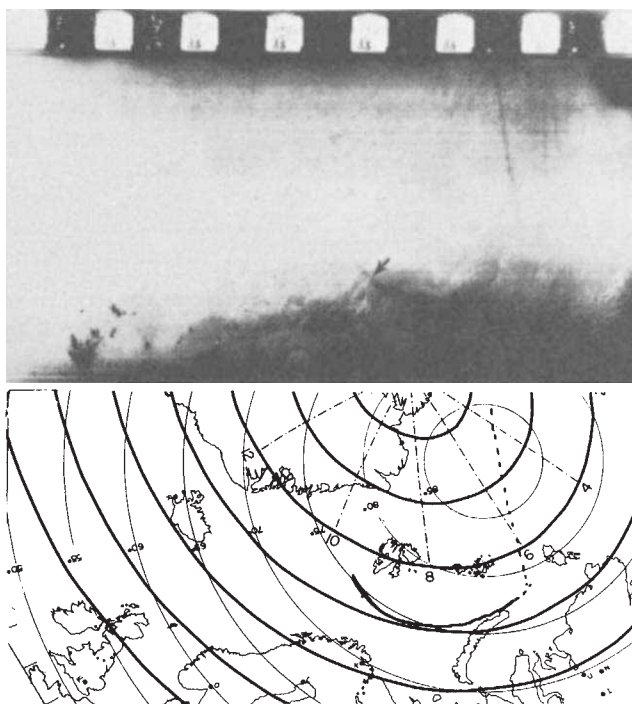


Fig. 2 DMSP photograph (in negative) taken at 2057 UT on December 24, 1972, together with an approximate geographic area covered by the photograph; it shows also the invariant latitude lines at 100 km altitude (thick lines) and geographic latitude lines (thin lines).



Fig. 3 DMSP photograph (in positive) taken at 1252 UT on January 12, 1973; see the caption for Fig. 2.

even when B_z has a large positive value for an extended period. During such a period, the auroral oval has the minimum area A_0 .

So the magnetospheric substorm can be considered as a process by which the magnetosphere tends to remove the excess energy ϵ_s associated with S . When the auroral oval at a particular time is significantly greater than its minimum size,

$$\epsilon_s \approx (L/R_1^2 V_1 \pi^4) S^2$$

where L is the length of the magnetotail, R_1 is the radius of the magnetotail and V_1 is the speed of the solar wind.

Figure 2 shows one of the smallest auroral ovals observed by the DMSP satellite. It was taken during a prolonged period of a large positive B_z value. Figure 3 shows an intense auroral substorm along an expanded medium size oval; this event occurred when the B_z component was negative. There is a considerable difference in the size of the oval and auroral activity, as expected from the above equation.

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Natural halocarbons in the air and in the sea

ORGANOCHLORINE compounds are usually taken to be man made; yet methyl chloride has been demonstrated as a product of microbial fermentation¹ and of the smouldering combustion of vegetation (A. E. O'Keeffe, personal communication) and Zafriou² has predicted its ubiquitous presence in the environment. Here I report measurements of methyl chloride and other halomethanes in the air and coastal waters of southern England between December 1974 and April 1975. These measurements confirm those of R. A. Rasmussen (personal communication) who found methyl chloride in rural air over the West Coast of the USA; they reveal methyl chloride as the dominant halocarbon of the atmosphere. The possible bearing of this discovery on the chlorine-catalysed destruction of stratospheric ozone is discussed.

Analysis was by gas chromatography using an electron capture detector which was operated at a temperature of 270 °C. This high temperature encouraged the otherwise sluggish reaction of methyl chloride with free electrons³. 5-ml air samples were analysed directly and water samples were analysed by taking 5-ml samples of nitrogen previously equilibrated with the water. The limit of detection for methyl chloride was 8.5×10^{-11} by volume.

Figure 1 compares the ambient concentration of methyl chloride and CCl_3F at Bowerchalke, southern England from

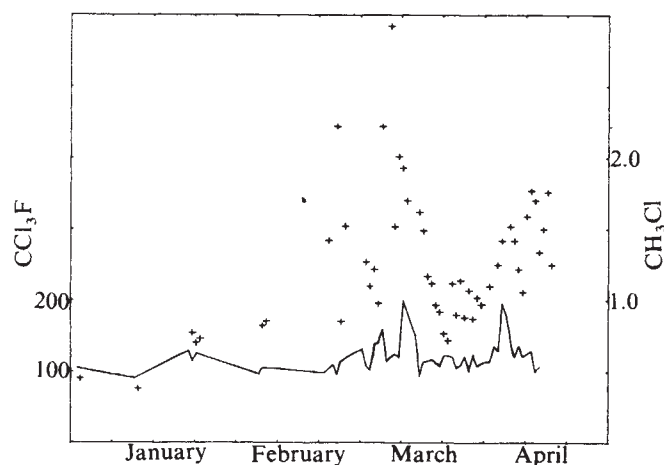


Fig. 1 Aerial concentration of CH_3Cl (+) and of CCl_3F (solid line) in parts per 10^9 and 10^{12} by volume respectively.

December 1974 until April 1975. The concentration of both gases varied with wind heading but methyl chloride was most abundant with air from the Atlantic whereas CCl_3F was most abundant with air from continental Europe. The mean concentration of methyl chloride over this period was 1.14×10^{-9} by volume. Table 1 lists the concentrations of halocarbons in seawater collected on the Kimmeridge ledges 30 miles south of the air sampling site. These inshore waters, rich in kelp, can be seen to carry large but variable amounts of halocarbons. Table 2 lists observations of methyl iodide in seawater during the voyages of the RV Shackleton in 1971 and 1972 and the RV Meteor in 1973. The first voyage covered the Northern and Southern Atlantic and Antarctic Oceans, the second the North Atlantic and the Caribbean Oceans. The table also includes some measurements of seawater concentrations of methyl iodide over kelp beds in

South-west Ireland. These latter measurements were during the summers of 1973 and 1974.

Methyl iodide has been found in all ocean waters so far examined and may be a common product of marine algae. The large kelp, particularly *Laminaria*, seem to specialise in the production of methyl iodide and water in the vicinity of the *Laminaria digitata* beds of South-west Ireland was found to contain one thousand times as much as is found in the open oceans. Other inshore algal beds, for example those of *Fucus*, showed no such increase. If methyl chloride is the product of the reaction between methyl iodide and the chloride ion of seawater², then the distribution of methyl chloride and perhaps also methyl bromide might be expected to follow a similar pattern to that of methyl iodide. So far only inshore waters have been examined for these compounds, where the concentration is so much greater than that which would be in equilibrium with the air that the sea is undoubtedly a strong source. If the halomethanes are the halogen gases discovered in the pioneering measurements of Duce and his colleagues^{4,5} then the methyl halides are indeed ubiquitous.

The rate of reaction of methyl chloride with OH in the troposphere is such⁶ that if this were the sole sink the residence time would be 0.37 yr. Applying this value to the observed mean concentration of 1.14×10^{-9} suggests a source strength of 28 Mt yr^{-1} . The industrial production of methyl chloride in 1973 was 0.35 Mt; this was used almost entirely as a chemical intermediate in the production of other compounds and did not reach the atmosphere as such. The atmospheric CH_3Cl is therefore almost certainly not a direct industrial emission. From the model introduced by Liss and Slater⁷ some estimates of the seawater concentration needed to sustain a flux of 28 Mt yr^{-1} to the atmosphere can be calculated. This would suggest that with an aerial concentration of 1.14×10^{-9} the mean seawater concentration would need to be $1.9 \times 10^{-8} \text{ ml of gas per ml of seawater}$. This requirement was reached on one occasion for the waters examined but an extended survey will be needed to determine the proportion of methyl chloride of marine origin. Approximately 1% of the chlorine content of vegetable matter is converted to methyl chloride during smouldering combustion. Grass and forest fires, stubble burning, and slash and burn agriculture are therefore potential sources also.

What is the significance of the high concentrations of methyl chloride for the present concern over the chlorine-catalysed destruction of stratospheric ozone? Unlike the freons, methyl chloride is comparatively unstable chemi-

Table 1 Halomethanes* in water from the seashore at Kimmeridge, Dorset, England

Date	Water temperature (°C)	CH_3Cl	$\text{CH}_3\text{Br}^\ddagger$	CH_3I
12.1.75	45	7.2 (2.7)†	2.0 (0.7)	1.3 (0.3)
8.3.75	42	5.9 (0.8)	1.5 (0.30)	1.2 (0.3)
9.4.75	42	21 (12)	3.9 (2.0)	2.8 (0.7)

*Concentrations are in ml of gas per ml of water $\times 10^{-9}$.

†Figures in brackets are standard deviations.

‡ CH_3Br confirmed by retention time only.

Table 2 Methyl iodide in surface seawater

Date	Site	Concentration*
1971-72	Open ocean Atlantic and Antarctic	135 (248)
1973	Open ocean Atlantic and Caribbean	138 (47)
1973	SW Ireland	$3.4 (1.8) \times 10^3$
1973	Kelp beds SW Ireland	$1.2 (0.9) \times 10^5$

*Concentrations as ml of vapour per ml of water $\times 10^{-12}$. Figures in brackets are standard deviations.

cally and the greater part of it would be destroyed in the troposphere. But methyl chloride has a source which is globally distributed and a residence time long enough to permit mixing in the troposphere. In these circumstances the observed ground level concentrations are likely to be representative and extend to the tropical tropopause. The proportion of chlorine conveyed to the stratosphere as methyl chloride to that conveyed as freon molecules will therefore be as their concentrations at the tropical tropopause. If other chlorine compounds are included, the present estimates suggest that less than 20% of the chlorine entering the stratosphere is in the form of freon⁶. Newell⁸ calculates that the residence time for a molecule in the stratosphere between the 10 and 100 mbar levels is 2 yr. During this time nearly all of the methyl chloride entering the stratosphere will have been destroyed, releasing its chlorine. Because of their much greater stability the most likely fate of freon molecules entering the stratosphere is to return back to the troposphere. When this additional factor is taken into account the present production of chlorine in the stratosphere is mostly from naturally occurring methyl chloride. If the freons are allowed to accumulate in the atmosphere without hindrance eventually their concentration may reach levels which are hazardous. But at present they seem to constitute only a minor stratospheric chlorine source.

It is reasonable to assume that any man-made perturbation of a major compartment of the atmosphere such as the stratosphere is at the very least undesirable. In this debate, however, it is always assumed that ozone depletion alone is the serious problem and potential threat; the possible dangers of ozone accretion are in this context considered as irrelevant. An alternative view of the significance of odd nitrogen and odd chlorine in the atmosphere comes from considering the possibility that the cycling of gases by the biosphere is not merely passive but represents an active process concerned with homeostasis⁹. In the context of this hypothesis the large scale biosynthesis of methyl chloride and nitrous oxide, and the lesser production of the more potent methyl bromide, suggests a natural process for ozone depletion. Could it be that the biosynthesis of these compounds responds to some function of stratospheric ozone density and acts as a regulator?

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Terrain units in eastern Antarctica

APPROXIMATELY 12,000,000 km² of bedrock in Antarctica is inaccessible, except to remote sensing, beneath an ice sheet up to 4.5 km thick. The topographical and geological pattern of the subglacial continent has, consequently, been little investigated and no more than a few geophysical measurements have been obtained. The availability of continuous records of bedrock topography from airborne radio echo sounding, however, now

facilitates the calculation of statistics of terrain roughness, which can be used quantitatively to differentiate subglacial regions, to cluster similar units, and to indicate associations or characteristics related to geological factors¹.

A joint programme between the Scott Polar Research Institute (SPRI) and the US National Science Foundation (NSF) has obtained over 300,000 km of profiles in Antarctica since 1967 using pulse-modulated radar operating in the frequency range 35–60 MHz (refs 2–6). Some of these results have been used to define and interpret terrain units in eastern Antarctica.

Information on terrain roughness is available at two scales. A qualitative examination of detailed bedrock relief maps (contour interval 250 m) compiled following radio echo exploration (SPRI Antarctica Radio Echo Sounding Map Series A, sheet 3, 1974; scale 1 : 5,000,000) allows the delimitation of major physiographic provinces⁷ (Fig. 1). Quantitative measures of the fine structure of surface roughness can be obtained from statistical analysis of the variation of relief within macroscale regions.

Vertical and horizontal variations in bedrock elevations have been calculated by the use of two simple statistics—the dispersion of elevations about the mean normalised to zero (D , standard deviation) and the autocorrelation distance (R_{xd}) calculated from the normalised autocorrelation function (with zero mean):

$$R_x = (n/n-k) \left[\left(\sum_{x=1}^{n-k} V_x \times V_{x+k} \right) / \sum_{x=1}^n V_x^2 \right]$$

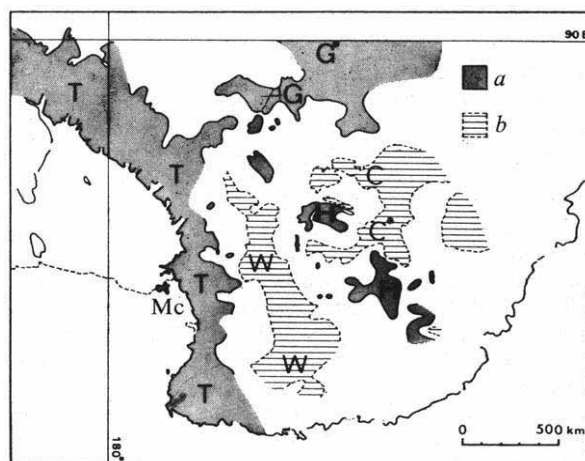
where V_x = discrete bedrock elevation; n = total number of elevations; k = lag of unit 1.5 km (the sample interval).

R_{xd} is defined as the lag to the j th % confidence level, usually taken as 95%. It is a distance over which roughness shows little or no statistical variation within given limits.

D and R_{xd} were computed for radio echo profile elevation data within macroscale regions of eastern Antarctica (Fig. 2). Two topographic groups can be identified, one comprising lowland areas with D values < 180 m (the Wilkes Basin and the basin in central eastern Antarctica) and a second, less homogeneous group of highland regions with values of $D \geq 200$ m (the Gamburtsev Mountains, subglacial portions of the Transantarctic Mountains and the massif in central eastern Antarctica).

The Gamburtsev Mountains fall into two distinct parts: one

Fig. 1 Major large-scale terrain regions of eastern Antarctica. T, Transantarctic Mountains; G and G*, Gamburtsev Mountains; H, highland massifs within central eastern Antarctica; W, Wilkes Basin; C and C*, central basin of eastern Antarctica; Mc, McMurdo Sound; a, terrain over 250 m; b, terrain below 500 m. (Both relate to present sea level.)



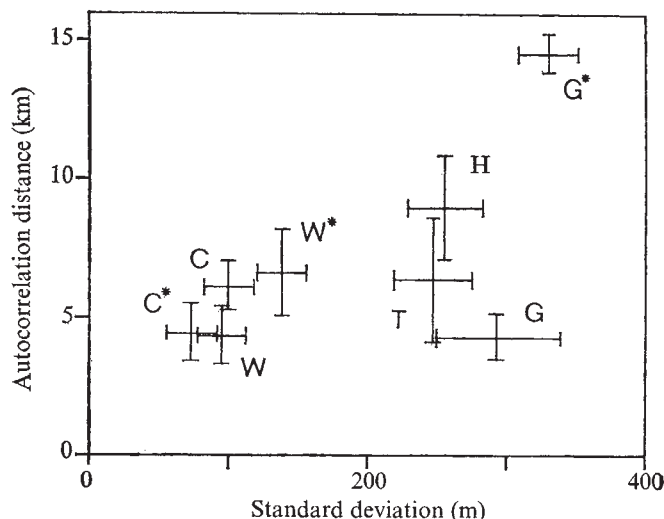


Fig. 2 Average terrain roughness characteristics of topographic units of eastern Antarctica. Bars indicate root mean squares of measurements. G and G*, Gamburtsev Mountains; H, central massif in eastern Antarctica; T, Transantarctic Mountains; W, central portion of Wilkes Basin; W*, marginal zones of Wilkes Basin; C*, northerly portion of central eastern Antarctic Basin; C, southerly portion of central eastern Antarctic Basin.

has a very high R_{xd} value indicating a smoother, more regular topographic surface, but is dissected by deep valleys to yield a commensurately high D value; the other, with a low autocorrelation distance, is typical of very rugged, rough mountainous terrain: values for D and R_{xd} are comparable with similar statistics obtained from other high latitude 'alpine' areas (for example, Alaskan ranges)¹.

Such contrasts within contiguous mountain zones probably originate from differences in the geological pattern. Unfortunately, no supporting geophysical information is available for this region. Nevertheless, it has been suggested that the Gamburtsev Mountains may have formed part of a large geosynclinal series deformed during a late Proterozoic-early Phanerozoic orogeny; an early Proterozoic deformational event has also been proposed¹. The presence, however, of at least two distinct terrain provinces suggests a more complicated history of block formation and uplift. It may be that periods of epeirogenic reactivation in the Gamburtsev Mountains did not have uniform effects, either in time or place, leading to later diversification of terrain. The same result could also be the product of the differential stripping of an epiplatform cover developed during the Phanerozoic.

The Transantarctic Mountains and massifs within central eastern Antarctica show statistical similarities, but both are quite different from the Gamburtsev Mountains. The structural control of relief in both the exposed and the subglacial parts of the Transantarctic Mountains is partially attributable to the presence of the sub-horizontal sediments of the Beacon Supergroup and associated intrusive bodies^{1,9,10}. Values of terrain roughness indicate that the central massif also falls within the same province of Phanerozoic sedimentation. Smaller R_{xd} values for the subglacial Transantarctic Mountains may be attributable to the more active erosional development of the mountains in the mid-Cainozoic, during the growth phase of the eastern Antarctic ice sheet¹¹, and to the later fluctuations of its margin in that zone¹².

The two major basinal areas of eastern Antarctica show strong similarities in terrain roughness, yet although they possess low D values they cannot be termed plain-like since they exhibit equally low R_{xd} characteristics.

The Wilkes Basin *sensu stricto* and its marginal areas were analysed separately. Results suggest that this depression (below present sea level), with only small scale surface irregularities along its axial core, is rimmed by a much rougher transition zone. Such

differentiation implies that the basin may contain a smoothing cover of Mesozoic and Cainozoic deposits which is absent from its flanks because of erosion. Calculations from isolated magnetic measurements over part of the Wilkes Basin provide limited evidence for the presence of a discontinuous, sedimentary sequence up to 6 km thick in places¹³.

Similar, but slightly less marked, differences exist between two portions of the central eastern Antarctic basin. Since the margins of this basin show no distinctive roughness characteristics compared with the Wilkes Basin, any differences may result from a slightly greater surface dissection of a sedimentary mantle in the northern sector of the basin though it cannot be positively stated that sediments line the depression because of the absence of geophysical observations. In a small isolated basin beneath the Soviet Vostok base, 300 km to the south, however, seismic investigations of the upper crust indicate a thin layer of sediments¹⁴.

These analyses may be important aids to the geological interpretation of inland areas of Antarctica and could be used as a primary reconnaissance tool for the location of critical areas worthy of detailed study using other geophysical techniques.

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Mechanochemical availability

THE thermodynamic functions 'availability' and 'maximum work', as they are usually defined¹⁻³, are generally inapplicable to systems that interest the biologist and electrochemist for the reasons shown below. Here I propose the redefinition and generalisation of both functions, and explicitly define a thermodynamic property, the 'mechanochemical availability', that provides a measure of the maximum usable mechanochemical work that can be released in the interaction of a contractile system with a source or sink of chemical potential of constant intensive state.

The 'maximum work' function

$$\Delta W \equiv \Delta U - T_s \Delta S \quad (1)$$

is the work input along the singular process path that takes a system adiabatically and reversibly (isentropically) from its initial temperature, T_{system} , to the temperature of an interacting isothermal reservoir, T_s , (process l-c) and that has all

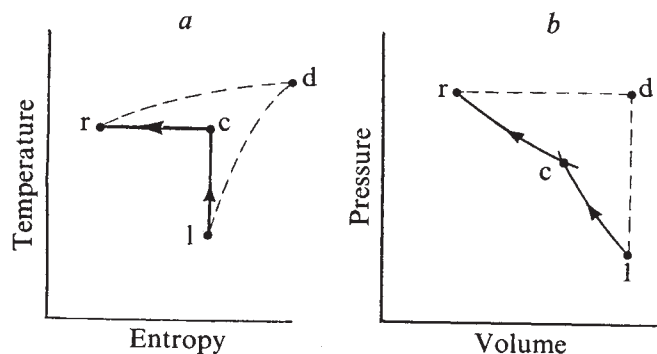


Fig. 1 T_1 may be greater than or less than T_r , but in this figure $T_1 < T_r$.

heat exchanges occurring subsequently and reversibly at T_r (process c-r) (Fig. 1a and b).

Thus

$$W_{1cr} = \Delta U_{1r} - Q_{1cr} = \Delta U - T_r \Delta S \equiv \Delta W \quad (2)$$

The 'availability' is defined as

$$\Delta B \equiv \Delta W + P_r \Delta V \equiv \Delta U + P_r \Delta V - T_r \Delta S \quad (3)$$

When a system is capable of exchanging several work modes with its environment, equations (1) and (3) are of limited use or interest. To illustrate this deficiency, consider that an open mechanochemical system can engage in the following reversible work modes

$$W = \sum_i W_i = - \int_1^r P dV + \int_1^r f dL + \int_1^r \sum_i \mu_i dn_i \quad (4)$$

which in turn equals

$$\Delta U - T_r \Delta S \equiv \Delta W \quad (5)$$

when the system is constrained so as to transfer heat reversibly at T_r .

If the system is further constrained to operate at constant P_r , then

$$\Delta W = -P_r \Delta V + \int_1^r f dL + \int_1^r \sum_i \mu_i dn_i \quad (6)$$

and

$$\Delta B = \int_1^r f dL + \int_1^r \sum_i \mu_i dn_i \quad (7)$$

Equations (6) and (7) are deficient because they fail to separate the contraction work, $\int f dL$, from the chemical potential interchange, $\int \sum \mu_i dn_i$. Only the former term measures the useful mechanical work exchanged with the environment, yet the magnitude of this term is completely obscured in equations (6) and (7). In a mechanochemical system the 'availability of contraction work' is the significant or relevant quantity.

This deficiency may be removed by defining ΔW more generally:

" ΔW is the work input along that combination of reversible process paths which brings a system from its initial equilibrium state to a final state in equilibrium with an interacting reservoir of fixed state, said paths being such that all heat transfer occurs

at T_r and all matter transfer occurs at the potential of the reservoir (μ_{ri} or V_r)."

It now follows that the work input to a system so constrained is

$$\begin{aligned} W &= \Delta U - Q = \Delta U - T_r \Delta S \\ &= - \int_1^r P dV + \int_1^r f dL + \sum_i \mu_{ri} \Delta n_i \end{aligned} \quad (8)$$

As a further consequence, the 'maximum mechanochemical work' can now be defined as

$$\int_1^r f dL \equiv \Delta W_{mc} = \Delta U - T_r \Delta S + P_r \Delta V - \sum_i \mu_{ri} \Delta n_i \quad (9)$$

if the system operates at P_r , and the 'mechanochemical availability' may now be defined as

$$\Delta B_{mc} \equiv \Delta W_{mc} - f_r \Delta L = \int_1^r f dL - f_r \Delta L \quad (10)$$

or

$$\Delta B_{mc} \equiv \Delta U - T_r \Delta S + P_r \Delta V - \sum_i \mu_{ri} \Delta n_i - f_r \Delta L \quad (11)$$

The significance of equations (9)–(11) is illustrated by Figs 2 and 3 which show the computation paths for ΔW_{mc} and

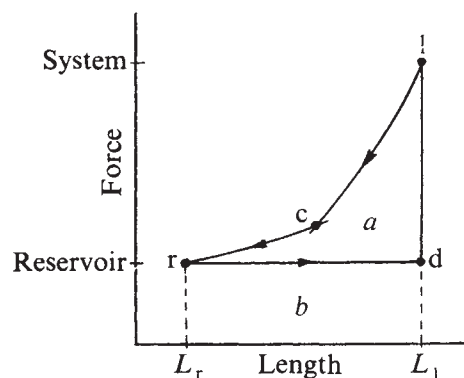


Fig. 2 Area $a = \Delta B_{mc}$; area $b = -f_r \Delta L$.

ΔB_{mc} , where 1 is any initial state of the system and r is the state of the system when in equilibrium with an interacting reservoir at T_r , P_r , μ_{ri} and f_r . Process 1-c is an isomolar reversible process which reduces the chemical potential of the system reversibly from μ_1 to μ_r . Process (c-r) then allows mass transfer at constant μ_r .

As can be seen from equations (10) or (11) the area (1-c-r-d-1) is equal to the mechanochemical availability.

At constant T_r and P_r , the cyclic integral of the Gibbs equation for a mechanochemical system:

$$\oint dU = T_r \oint dS - P_r \oint dV + \oint f dL + \oint \sum_i \mu_i dn_i \quad (12)$$

reduces to

$$\oint f dL = - \oint \sum_i \mu_i dn_i \quad (13)$$

from which it follows that area (1-c-r-d-1) in Fig. 2 equals

the negative of area (1-c-r-d-1) in Fig. 3, if only one component is exchanged with the reservoir, and

$$\sum_i [\text{areas } (1-c-r-d-1)_i]$$

if i components are exchanged.

An analogous representation of the ΔB of equation (3), that is, the availability in a thermally activated expansion work system, is shown by the cycles (1-c-r-d-1) in Figs 1 and 2. The ΔB cycles always involve the properties of the work coupling and potential terms that appear in the Maxwell relations for the system. For a thermally activated expansion work system the relevant Maxwell relations in Jacobian form⁴ are

$$[P, V] = [T, S] \quad (14)$$

whereas for a mechanochemical system at constant T and P they are

$$[f, L, T, P] = -[\mu_i, n_i, T, P] \quad (15)$$

The graphical representation of ΔB always consists of a cycle in which a constant n_i (or S) process is followed by a constant μ_i (or T) process, with the cycle completed by constant P and constant V processes or constant f and constant L processes. The area of the cycle is always less than or equal to zero.

By analogy the electrochemical availability may be defined as

$$\Delta B_{ec} \equiv \Delta U - T_r \Delta S + P_r \Delta V - \sum_i \mu_{ri} \Delta n_i - V_r \Delta q \quad (16)$$

where V and q are voltage and charge, and a 'general availability function' may be defined as

$$\Delta B_g \equiv \Delta U - T_r \Delta S + P_r \Delta V - \sum_i \mu_{ri} \Delta n_i - f_r \Delta L + \sum_i F_{ri} \Delta X_i \quad (17)$$

where $\sum_i F_{ri} \Delta X_i$ represents the sum of all other reversible work forms expended against the interacting reservoir.

Is path (1-c-r) the maximum work path?

Curve (1-k-r) in Fig. 4 represents an arbitrary reversible mechanochemical process connecting the initial state (1) to the reservoir state (r). The area under (1-k-r) is clearly greater than area under the ΔW path (1-c-r).

Path (1-a-b-r), consisting of isomolar processes (1-a) and (b-r) and isopotential process (a-b), is so drawn that the area under (1-a-b-r) is equal to that under (1-k-r) (Clausius' theorem⁶).

For a constant temperature-constant pressure reversible

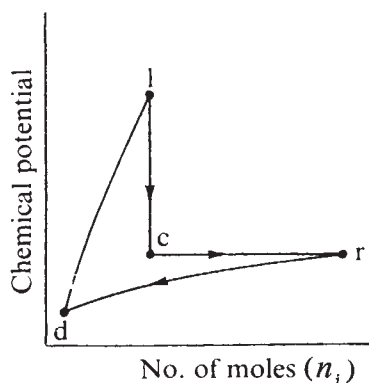


Fig. 3

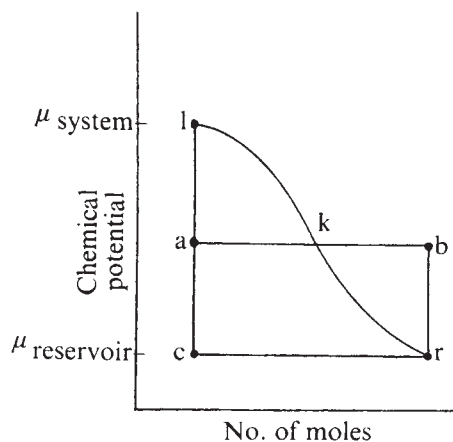


Fig. 4

mechanochemical process whereby a system at 1 interacts reversibly with a reservoir at r

$$\int_1^r dU = T_r \int_1^r dS - P_r \int_1^r dV + \int_1^r f dL + \int_1^r \sum_i \mu_i dn_i \quad (18)$$

or

$$\int_1^r f dL = \Delta U_{1r} - T_r \Delta S_{1r} + P_r \Delta V_{1r} - \int_1^r \sum_i \mu_i dn_i \quad (19)$$

It follows from equation (19) that if the $\int \mu_i dn_i$ along path (1-k-r) is equal to the integral along (1-a-b-r), then the contraction works

$$\int_1^r f dL$$

for both paths are also equal. Now the work of process (1-a-b-r) exceeds that of process (1-c-r) by

$$\sum_i \left[\text{area } (a-b-r-c-a)_i \right]$$

For process (1-a-b-r) to occur reversibly, however, it must absorb chemical work ($\mu_i dn_i$) at μ_b which is greater than the μ_r of the environment. A Carnot chemical pump⁶ (the chemical work analogue of a heat pump) will therefore be needed to transfer this chemical work from the μ_r to the μ_b reversibly. The work input to this pump(s) is exactly equal to

$$\sum_i \left[\text{area } (a-b-r-c-a)_i \right]$$

so that the net reversible work obtainable from the system (1) interacting with reservoir (r) is $(-)$ the area under (1-c-r) plus $(\Delta U - T_r \Delta S + P_r \Delta V)$, or $\int f dL$ along path (1-c-r).

Note added in proof: I thank Professor J. Keenan for pointing out that equation (9) is analogous to equation (55) of ref. 7.

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Wet preparation of 'water glass' from the hull ash of Egyptian rice

THE FAO and other organisations have suggested useful applications for the rice hull in agriculture¹. But promotion of the use of the material for industrial applications was ignored. The use of the hull and its ash in the preparation of furfural and ceramic products has now been shown to be possible¹, but only the preparation of furfural from the hull has been established (in the USA and Italy) as a sound industrial process. Nevertheless, the furfural industry consumes only a small fraction of the material available, and research into other potential applications is obviously desirable. The work described here is an attempt at the wet preparation of water glass by decomposing the hull ash with sodium hydroxide solution at a moderately high temperature.

Water glass is normally prepared by fusing the appropriate quantities of sodium carbonate and sand at a temperature around 1,400 °C to produce a soda-silica glass. This is converted into a viscous liquid through the action of water vapour under pressure². The material has many applications in detergents, adhesives, ceramics, paper, textiles and other industries.

The annual world production of rice is $\sim 250 \times 10^6$ t, of which about 10^6 t are produced in Egypt. The hull constitutes about 20% of the rice grains. The material has a very low bulk density (about 0.13 g cm⁻³), and thus occupies

Table 1 Chemical analysis of the Egyptian rice hull ash

Constituent	Weight (%)
SiO ₂	91.28
TiO ₂	0.12
Al ₂ O ₃	2.82
Fe ₂ O ₃	0.48
CaO	1.11
MgO	0.85
Na ₂ O	0.47
K ₂ O	1.79
Loss on ignition	0.23
Total	99.33

a very large space. It also has a high silica content which makes it of little value as food for animals. The hull is used as a fuel, especially in some rice mills. When well burnt it leaves a whitish fluffy voluminous ash which retains the original shape of the hull, and consists chiefly of silica in an amorphous form¹.

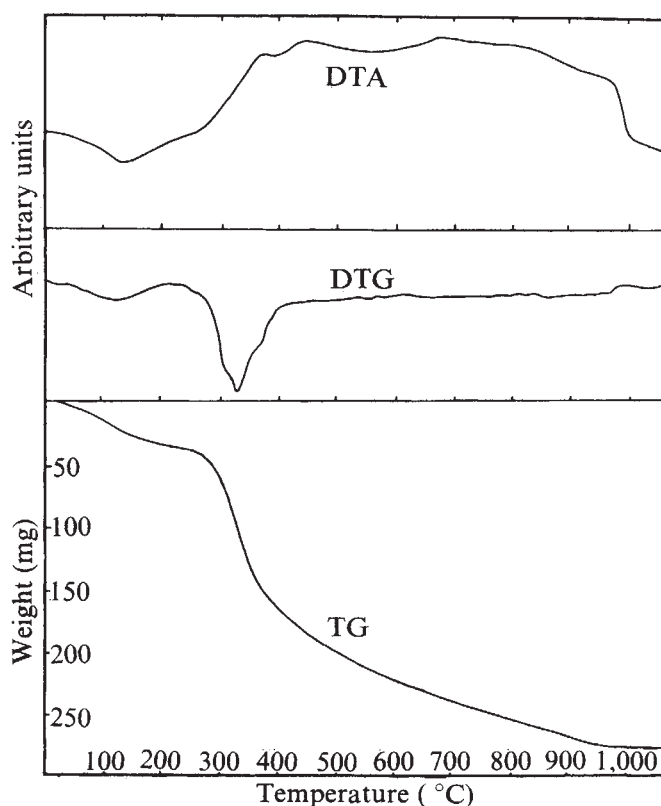
For the determination of the ash content of the hull and the thermal behaviour during ignition, thermogravimetric (TG), differential TG and differential thermal analysis (DTA) graphs were established on a Model OD-102 Hungarian-made Derivatograph.

Figure 1 illustrates TG, DTG and DTA curves for a 340-mg sample of the hull. The sample was heated from room temperature to 1,020 °C in an atmosphere of air at a heating rate of 10 °C min⁻¹, using alumina as the inert reference. The DTA curve shows an initial endothermic peak at about 130 °C corresponding to the loss of adsorbed water. This is accompanied by a similar endothermic DTG peak at a slightly lower temperature (115 °C), and a loss in weight (represented by the TG curve) of about 4.4% of the initial sample weight. The thermal decomposition of the hull is characterised by two exothermic DTA peaks at about 350–400 °C and a composite endothermic DTG peak with the principal apex at 330 °C. These peaks are probably characteristic of cellulose and hemicellulose which are the major constituents of the organic part of the hull. Corresponding to these reactions there was an abrupt loss in weight of about 36.6% of the initial sample weight. The decomposition of the remaining part of the hull was gradual and was completed at about 942 °C after which the weight of the residual ash became fixed at 60 mg (about 18% of the original sample weight).

Complete chemical analysis of the ash remaining after the complete burning of the rice hull was carried out by the method described by Riley³. Table 1 presents the percentages of its various constituents, the main one being silica (91.28%). On washing the ash with 0.5 N HCl overnight the silica content rose from 91.28% to about 95.5%. This is chiefly because of the preferential leaching of alkalis and other basic constituents in the form of chlorides.

The decomposition of the ash was carried out in a bomb of 250 ml capacity, made of stainless steel and placed in a thermostatically controlled kiln. In each experiment 5 ml of distilled water was mixed with 5.0 g of a mixture of ash and sodium hydroxide, which had a silica-soda ratio of 2:1. The bomb containing the mixture was then placed in the kiln, which was previously adjusted to the required temperature, for the required time. After the experiment was completed, the bomb was taken out of the kiln, cooled to room temperature and opened. The contents were then discharged in a 250-ml beaker, using 30–40 ml water and quickly filtered through sintered glass. For determining the degree of decomposition of the ash, the filtrate was analysed for its silica content. Figure 2 illustrates the time dependence of decomposition at 100, 125, 150, 175 and 225 °C. The fraction decomposed increases with time and temperature, the reaction being fast in its early stages and slower as complete decomposition

Fig. 1 Ash content of the hull of Egyptian rice.



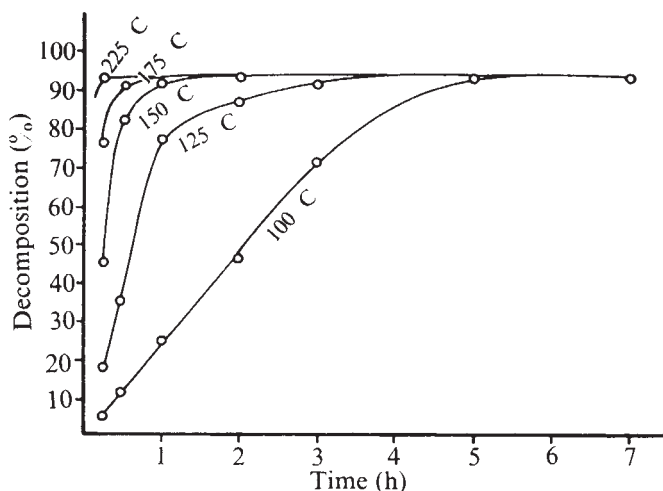


Fig. 2 Time dependence of decomposition of the raw ash at different temperatures.

is approached. Nearly complete decomposition is achieved after 5, 4, 2, 1 and less than 0.5 h at temperatures of 100, 125, 150, 175 and 225 °C, respectively.

For comparison raw ash, acid washed ash and finely powdered quartz and fused silica (–150 mesh) were subjected to a decomposition experiment at 150 °C for 1 h. The results are given in Table 2. The raw ash is much more easily decomposed than the other forms of silica. The acid treated ash is even more easily decomposed. This can be attributed to the partial leaching of cations such as Al^{3+} , Ca^{2+} and Mg^{2+} , which are known to suppress the dissolution of silica in alkali solutions by precipitating it as silicates⁴.

The explanation of the decomposition of the ash by NaOH, can be simplified by taking the relatively simple structure of pure silica as a first approximate model for the more complicated structure of the ash. The effect of other cations can be treated separately.

In all forms of silica, silicon is known to be tetrahedrally coordinated to four oxygen atoms. Within the material, the coordination of oxygen and silicon of the tetrahedral units is satisfied. At the bounding surface, however, silica may expose incomplete tetrahedra and the surface ions will strive to complete their coordination. In contact with aqueous solutions the exposed ions will add hydrogen or hydroxyl ions so that the surface is ultimately covered with Si–OH groups. The hydrogen of the Si–OH group is ionisable and may, in favourable conditions, leave the silica surface in a negatively charged state. According to the details of the structure, there are four ways in which these Si–OH groups may be exposed at the surface^{5,6} (Fig. 3). These four types show enormous variations in resisting attack by aqueous solutions. Although type I is very unstable in the presence of water, type IV is completely unreactive to alkalis⁵. A demonstration of the importance of the surface structure is provided by low quartz. The basal plane shows a rate of attack by aqueous solu-

tions a hundredfold greater than the prism faces. The former is known to present type II exposure whereas the latter alternates between type II and type III, chiefly existing in the more resistant type III (refs 5 and 6). Non-crystalline silicates such as glasses and rice hull ash would be expected to present surfaces in which the four structural types exist.

In the rice hull, cellulose, hemicellulose and other carbonaceous material, which interlink the structural silicate units, are converted into CO_2 and H_2O by burning. As these materials constitute some 80% of the hull, the ash would be expected to be left in a state of polymerisation from which it can be more easily decomposed than pure forms of silica. This is, indeed, the case (Table 2).

In fact, simple shaking of ash with water at room temperature for a few minutes, brought about the maximum solubility of silica in water (about 0.01%). This supports the view that the structural units in the ash contain a high proportion of the easily soluble monomeric and dimeric units of silica.

The presence of polyvalent cations is known to suppress the dissolution of silica in alkaline solutions⁴. This is perhaps the reason why the maximum decomposition

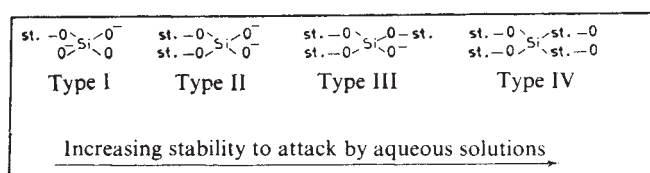


Fig. 3 The four possible exposures of the silica tetrahedra. st. = Structure.

achieved was about 94%. An experiment carried out on ash which has been previously washed with 0.5 N HCl, to remove some of the polyvalent cations, gave a maximum degree of decomposition of about 97% in the same conditions (see Table 2).

Thus, the structural state of the rice hull ash makes it superior to other forms of silica in the wet preparation of water glass. The economy of the process lies mainly in the relatively lower temperature at which reaction proceeds. Moreover, the hull—an inconvenient byproduct—can find a useful industrial application.

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Table 2 Decomposition of raw ash, acid washed ash and finely powdered quartz and fused silica with NaOH solution under pressure

Type of silica	Ratio of $\text{SiO}_2\text{--Na}_2\text{O}$	Ratio of water–solids	Temperature (°C)	Time (h)	Decomposition (%)
Raw ash	2:1	1:1	150	1	92.7
Acid washed ash	2:1	1:1	150	1	96.8
Quartz	2:1	1:1	150	1	7.0
Fused silica	2:1	1:1	150	1	60.7

Is the vegetation of continental Antarctica predominantly aquatic?

WE have suggested¹ that Antarctic lakes offer a more favourable physical environment to certain species of moss than the surrounding land. We now present evidence in

support of the wider thesis that in certain areas of Antarctica most of the plant biomass occurs in aquatic habitats. In the austral summer 1973–74 we found more mosses and algae growing aquatically than terrestrially in the Ablation Valley area of Alexander Island (Fig. 1). The area is relatively free from ice and has a climate similar to the inland ice-free areas of continental Antarctica², although conditions are not quite so arid because of frequent intrusions of oceanic weather systems from the Bellinghausen Sea. Extensive terrestrial plant cover was, however, restricted to seven discrete patches of moss, lichen and algae, totalling 2,300 m² in about 40 km² of ice-free ground searched on foot. The patches were on north-facing slopes where groundwater welled up continuously during the short summer. Widely scattered, very small moss cushions also occurred between and beneath stones in a few other damp and wet places. The availability of water and soil instability seemed to be the two most important factors restricting terrestrial plant distribution.

Many small pools, free from ice in summer, lay on ice-cored unstable moraines which bordered George VI Sound (Fig. 1). Although many pools may exist for only a few years, 95% supported a rich algal flora and associated microfauna including the calanoid copepod *Pseudoboeckella* sp.. A few pools also contained moss.

Ponds, 500–10,000 m² in area, lay between the scree-clad mountains and the moraines of ice shelf of the Sound. They became partly or completely free from ice for a short period in summer. The turbidity of the waters varied from less than 10 to more than 90% transmission per m as a result of the suspended glacial clay. In all ponds with a light transmission of more than 50% m⁻¹, luxuriant moss growth covered large areas from 0.5 to 9.0 m depth. Stems grew 30 cm in length. *Campylopus polygamus* and *Dicranella* sp. were dominant. *Distichium capillaceum*, *Bryum algens* and another *Bryum* sp. were also found. Cover was estimated during SCUBA dives and varied from 40 to 80% in the 0.5 to 5.0 m depth zone and 20 to 50% overall. Total moss cover in these clearer ponds was 3,500 m² in an area of 10,000 m². Most of the remaining area was covered by rich

algal felts growing up to 10 cm thick. Less luxuriant algal felts occurred in many of the more turbid ponds.

Three large (1.5–6.5 km²), deep (50 to more than 117 m), ice-dammed lakes lay in the main valleys. They were permanently frozen, ice cover ranging from 4.0 to 4.5 m thick in winter, 2.5 to 3.0 m thick in summer. No moss was seen in five dives covering about 10,000 m² of the bottom of the lake in Ablation Valley, and extending to 15 m depth. The only vegetation observed was a thin film of algae on the occasional rock jutting out of a silt floor. Silt-ing alone is unlikely to have prevented moss growth because the water contained less suspended clay than some of the ponds in which mosses were abundant. The dives were carried out near the shore where the ice was more than 5 m thick and snow cover persisted for most of the summer. This may have reduced light levels below that required for moss growth. Further out in the lake where the ice was snow free and thinner, light levels may have been insufficient due to the deeper water. Mosses may have been present in other areas of this 6.5 km² lake, particularly near the north-west shore where the snow cover disappeared very early in summer. It should be noted that mosses have been found at 35 m depth in other permanently frozen lakes³.

Contrary to our findings in the South Orkney Islands and South Georgia¹ mosses grew well in shallow water where ice scour could occur. Most of the moss must be frozen each winter. This must bring into doubt our earlier conclusion that mosses are unable to withstand ice scour. But the way ice cover melts on the Ablation ponds may differ so that ice scour is not a significant factor.

Further evidence for the predominance of aquatic vegetation in continental Antarctica is available. Rich benthic floras have been discovered in Lake Bonney⁴, South Victoria Land (77°S, 161°E), lakes in the Bunger Hills⁵ (66°S, 101°E) and in the Schirmachervatna⁵ (71°S, 11°E). These are very arid areas free from ice where terrestrial vegetation is extremely limited.

We conclude that in such areas the vegetation may largely be confined to aquatic habitats. Consequently biologists should look more closely at aquatic habitats when carrying out surveys in continental Antarctica.

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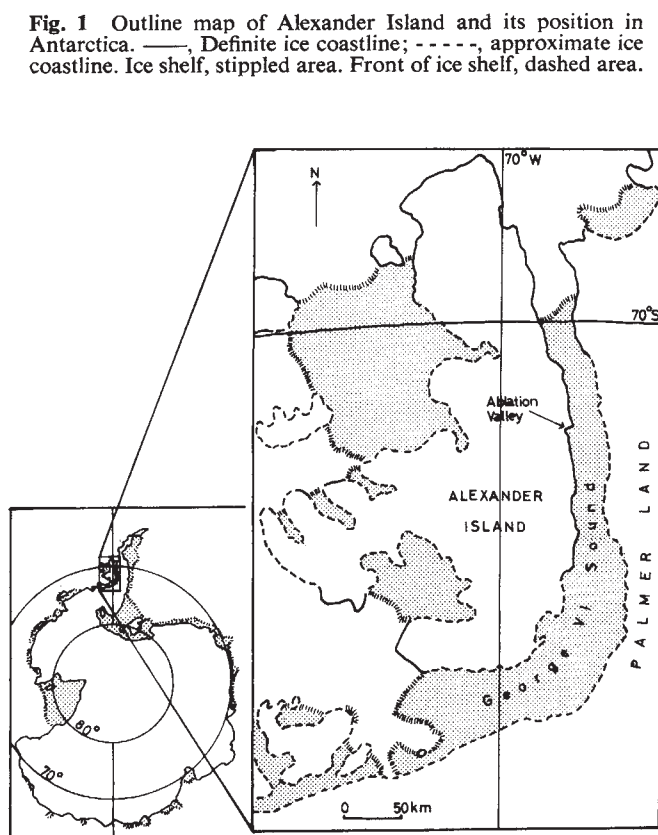


Fig. 1 Outline map of Alexander Island and its position in Antarctica. —, Definite ice coastline; - - - -, approximate ice coastline. Ice shelf, stippled area. Front of ice shelf, dashed area.

Form and function in corrugated insect wings

SELECTION is likely to have resulted in the evolution of insect wings which combine aerodynamic efficiency with a rotational moment of inertia about the wing base small enough to reduce as far as possible the energy expenditure involved in their repeated accelerations. Their construction has to leave them stiff enough to remain aerodynamically efficient when under inertial or aerodynamic load, and free from buckling, however light they become. Insect wings are very light structures—11 g m⁻² in the dragonfly *Aeschna cyanea*, 16.7 g m⁻² in *Locusta migratoria*¹ and 7.4 g m⁻² in *Tipula* sp.²

Hertel¹ has drawn attention to the corrugated chordwise profile of the anterior part of dragonfly wings, to which he attributed much of the wing stiffness, and commented on the

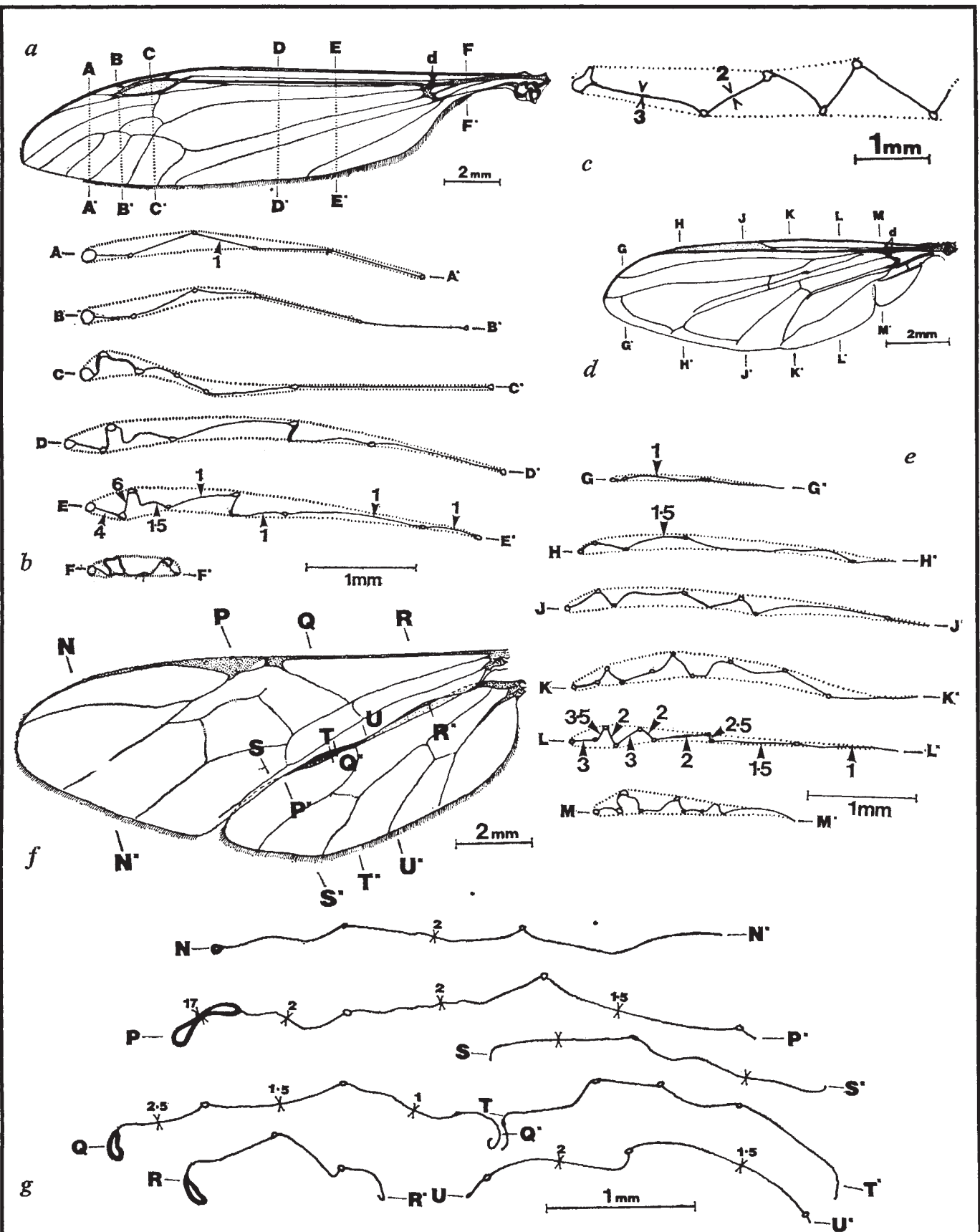


Fig 1 a, Left wing of *T. oleracea* (crane fly). d, Cuticular bar which reinforces the corrugation. Section letters apply to b. b, Chordwise sections of *T. oleracea* wing. Tubular veins to scale, membrane thickness shown diagrammatically. Some measured membrane thicknesses are shown (μm). Dotted lines, section envelopes. c, A section of the anterior portion of the chord of an *Aeschna cyanea* forewing (anisopteran dragonfly), redrawn from Hertel¹. d, Left wing of *Syrphus balteatus* (hoverfly). d, Cuticular bar reinforcing corrugation. Section letters apply to e. e, Chordwise sections of the wing shown in d. f, Left wings of *Ophion luteus* (ichneumon). g, Chordwise sections of the wings shown in f.

similarity between the disposition of the spars which form the web between upper and lower skins of an aeroplane wing, and the corrugated section of the *Aeschna* wing. In an aeroplane wing with a double skin separated by struts, however, it is the function of the struts to prevent the deformation of the smooth aerofoil profile presented by the skin, as is necessary at the higher Reynolds numbers appropriate to aircraft. In insect wings there is no enveloping 'skin' and any aerofoil properties must be sought in the corrugated wing sheet itself, operating at much lower Reynolds numbers at which the effect of corrugation on aerofoil lift and drag may not be so deleterious.

Figure 1 shows some series of chordwise sections of the wings of three insects: two dipterans (*T. oleracea* and *Syrphus balteatus*) and a hymenopteran (*Ophion luteus*). These were prepared by hand grinding thick slices cut from epoxy resin blocks, in which whole wings had been embedded, to approximately 10 μm thickness with wet carborundum paper. The embedding did not alter the corrugated form of the wings from their configuration in life; the corrugations were easily visible with either a stereomicroscope or a scanning electron microscope, where no embedding has been done. Corrugation is pronounced in both flies in most chordwise sections except those very near the wing tip. It tends to be deepest at about the one-third chord point from the leading edge, which has itself a notably rounded profile, especially in the *Ophion* wing. A somewhat similar architecture is seen in the wing of the beetle *Cicindela hybrida*³. Corrugation is less pronounced in *Ophion*, but the leading edge is well rounded, thickened and rigidly bent downwards.

Some comparisons of the mechanical properties of more regularly folded or tubularly reinforced beams may be relevant to a consideration of the load-bearing characteristics of insect wings. It is clear from Fig. 1 that insect wings are not beams of regular cross section, or of uniform width. They are corrugated beams of a sort, and I have therefore estimated some of the mechanical properties of a few beams of corrugated or tubulated cross section, the proportions of which are at least of the same order as those in some insect wings with respect to corrugation width, depth, membrane thickness and tube inner and outer radii. Experimental tests with metal scale models reveal that for small deflections corrugated beams which are loaded as cantilevers will behave as is predicted by simple beam-bending theory. Figure 2 summarises the dimensions of the beams which were compared, and Table 1 provides average

values of these dimensions for chordwise profiles of seven species of insects. It is hoped that these values will convey some impression of the relative cross sectional conformations of the wings of a diversity of insect species. Simple description of these profiles is otherwise difficult.

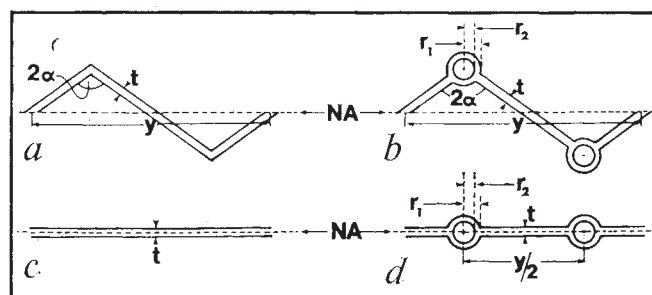


Fig. 2 a-d, Diagrams of parts of the cross sections of four beams the mechanical properties of which are compared in the text. a and b each show one corrugation repeat unit. NA, neutral bending axis. All other letters are those used in equations (1) to (4) in the text.

In making comparisons between regular beams, the following values were selected. The notation is as in Fig. 2, and the values apply to beams a-d in Fig. 2, as appropriate. Width of beams along neutral axis, $w = 1$ cm; length of beams $l = 1$ cm; $t = 1.2$ μm ; $2\alpha = 112^\circ$; $y = 0.05$ cm; $r_1 = 10$ μm ; $r_2 = 4$ μm . The beams were all assumed to be made of a material similar to insect cuticle with a density of $\rho = 1.1$ g cm⁻³ and Young's modulus $E = 10^{10}$ N m⁻² (ref. 4). Table 2 gives values of tip deflection D , maximum stress σ_M , second moment of cross sectional area I_x , mass m and rotational moment of inertia I_t about an axis passing through one end of the beam perpendicular to its length and parallel with the neutral axis and chord (Fig. 2).

The following relationships were used to compute the mechanical properties of the four beams a-d in Fig. 2. F is the force applied to the free end of the beams. $D = Fl^3/3EI_x$, $\sigma_M = Flz/I_x$, $m = \rho l A_x$ where z is the maximum distance of any part of the cross section from the neutral axis and A_x is the surface area of beam cross section. Second moments of cross

Table 1 Average values of beam dimensions for chordwise profiles in seven species of insect

Insect species	Average dimensions					Section position (mm)	
	y (cm)	t (μm)	2α (degrees)	r_1 (μm)	r_2 (μm)	A	B
<i>Aeschna cyanea</i> (dragonfly) forewing	0.15	2.5	102	60	50	12.8	0.27 *
<i>Chrysopa signata</i> (lacewing) hindwing	0.07	1.3	160	15	12	9.2	0.67
<i>Panorpa communis</i> (scorpion fly) forewing	0.03	1.0	150	20	16	10.8	0.77 †
<i>Arctia caja</i> (moth) forewing	0.40	5.0	146	70	60	25.0	0.86
<i>Ophion luteus</i> (ichneumon) forewing	0.15	2.0	158	15	12	2.6	0.37
<i>Tipula oleracea</i> (crane fly) forewing	0.10	1.5	128	20	17	15.7	0.81
<i>Syrphus balteatus</i> (hoverfly) forewing	0.05	1.2	112	10	4	3.9	0.41 ‡

*Averaged over the corrugations in the anterior third of the chord only.

†The tubular veins are here represented by veins of U-shaped section 40 μm deep and 32 μm wide.

‡These values were used in the comparisons of various regular beams. Let straight line chord length = C and number of veins in the section = N . Average y was estimated as $y = 2(C/N - 1)$, counting the trailing edge as a 'vein'.

Average t was that of the thickness of the inter-vein membranes, measured at the mid points (chordwise) of each.

A, Distance along span of chordwise section from wing root (mm); B, fraction of total wing length which A represents.

r_1 and r_2 were average outer and inner radii for all actual veins in the cross section. Where a vein is not circular in section, the average of its maximum and minimum radii was used.

2α represents the average angle included by all pairs of membranes where they merge with a vein.

Table 2 Parameters of regular beams

Beam section type	Deflection (D) (cm)†	Mass (m) (g)	Maximum stress (σ_M) (N m^{-2})	I_x (cm^4)	I_r (kg m^{-2})
a	0.97×10^{-3}	1.45×10^{-1}	2.48×10^5	3.43×10^{-9}	4.83×10^{-12}
b	0.32×10^{-3}	2.41×10^{-1}	8.64×10^4	1.03×10^{-8}	8.03×10^{-12}
c	$\geq 0.11^*$	1.32×10^{-1}	8.33×10^7	1.44×10^{-13}	4.40×10^{-12}
d	0.11*	2.37×10^{-1}	3.28×10^6	3.05×10^{-11}	7.90×10^{-12}

$$\text{where } I_r = \int_{r=0}^{r=l} \rho A_x r^2 dr = \frac{\rho A_x l^3}{3} = \frac{ml^2}{3} \quad (5)$$

*Deflections here will be increased by deformation under the beams' own weights, which will also increase the stress σ_M .

†Stress and deflection are expressed per 10^{-5} N applied at the free beam tip. The other ends of the beams are all fixed rigidly.

sectional area, I_x were obtained from the expressions:

For beam a:

$$I_x = \cot^3 \alpha / 48 (y^3 t \sec \alpha + 4y t^3 \sec^3 \alpha) w / y \quad (1)$$

For beam b:

$$I_x = \{ (h^3 / 3b^3) (c^4 + 4bc^3 + 6b^2c^2 + 4b^3c) + \pi [(r_1^4 - r_2^4) / 2 + 2d^2(r_1^2 - r_2^2)] \} w / y \dots \quad (2)$$

where $b = [(y \csc \alpha / 4) - r_1 - (t \tan \alpha / 2)] \csc \alpha$;

$h = b \sin \alpha \cos \alpha$; $c = t \sec \alpha$.

For beam c:

$$I_x = wt^3 / 12 \quad (3)$$

from Roark⁵, and for beam d:

$$I_x = \left[\frac{(y - 4r_1)t^3}{12} + \frac{\pi(r_1^4 - r_2^4)}{2} \right] \frac{w}{y} \quad (4)$$

We thus see that the introduction of corrugation is associated with scarcely any weight penalty (comparing beams a and c) although there are immense reductions both in deflection and in the maximum stress experienced for a given loading. The tubes, when added to the beam of corrugated cross section, increase its mass by a factor of 1.66, but result in a decrease in maximum stress by a factor of 2.87. Inclusion of tubes in a plane section (beam d) results in almost as great a mass as in the corrugated, tubed section (beam b), but beam d still deflects 337 times as far as beam b for a given end load, is 38 times as highly maximally stressed and yet its rotational moment of inertia I_r is only 2% less than that of beam d. What then is the function of the tubes? In a real insect wing, these tubes (veins or nervures) often carry haemolymph or serve as conduits for nerves and tracheae. In a corrugated beam with a very thin web there is in bending a tendency to collapse suddenly and catastrophically by buckling at the folds. The inclusion of tubes at these positions reduces the likelihood of this form of failure.

Insect wings show irregular depth of corrugation, both chordwise and spanwise. Corrugation tends to be deeper in the anterior half of the wing, and probably reflects the chordwise distribution of aerodynamic forces. In the use of corrugated structures, there is also the problem of preventing the folds from opening out when under load. In both fly wings in Fig. 1a and d there are stout chordwise bars (d) at the thoracic end of the corrugations which rigidly connect them in much the same manner as the end diaphragms in a corrugated roof to a building⁶. At the outboard end, as is seen in sections A-A¹ or G-G¹, the corrugation is hardly detectable. As such a nearly flat profile can permit virtually no further extension in a chordwise direction,

any corrugation in the wing between this flat region and the more proximal diaphragm (d) is made unable to flatten out, and the wing should retain its stiffness.

Unlike the double-skin aircraft wing, supported internally by a corrugated strut system, however, plainly corrugated wings like those of insects will have very little resistance to torsional deformation. Norberg⁷ has shown, however, in dragonflies that some torsional pitching of the wing during acceleration may allow increased flight speeds, under the influence of the pitching moment of the pterostigma, and that the natural axis about which the wing twists most easily is not far from the axis of fore-and-aft mass balance of the wing. Torsional deformation of inertial and aerodynamic origin may well be very small.

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Improving one's touch

DRAWING the finger tips across a smooth surface usually induces sensations of pressure, warmth or cold, and roughness or smoothness. Any abrupt discontinuity is easily felt: it has been claimed that eminences no higher than 0.001 mm etched on to smooth glass can be detected, provided some movement of the finger tips is permitted¹. We describe here an effect which does not seem to have been reported previously, even though we think that it must be familiar to certain craftsmen.

The effect is easily demonstrated. First, rub the fingers of one or both hands back and forth across a fairly smooth surface, such as polished wood. Note the accompanying tactile sensations. Next, place a piece of paper under the fingers and rub the surface as before, moving the paper with the fingers. It will be found that any gradual undulations in the surface which were hitherto undetected are now perceptible. (With practice and concentration it becomes possible to detect similar undulations without using the paper, but this is seldom as quick and easy.)

We have examined the effect in experimental conditions. A series of steel blocks, each 7.6 × 7.6 cm, was used. The upper surface of each block was ground smooth and flat and contained a central, raised, rectangular strip 3 mm wide. The height of the central strip varied from block to block. The central strip on each block was converted to a

gradual undulation by covering the upper surface with smooth card (0.5 mm thick) secured firmly at the edges.

Eleven subjects attempted to detect the orientation of the covered central strips by rubbing the surfaces with the fingers of one hand. On half the trials fingers alone were used, on the remaining trials a piece of writing paper was held under the fingers. The use of fingers alone compared with paper was counter-balanced across trials, as was the initial orientation of the hidden strip. Rubbing movements were made towards and away from the body; the subject was allowed to rotate the block through 90° whenever he chose. The subjects could not see the surfaces they were feeling. Twelve trials were carried out for each condition.

Performance at this task was so good, particularly when the paper was used, that we shall describe only the results obtained using the most difficult block, in which the central strip was 0.0127 mm high. Without the masking paper the orientation of the central strip on this block was correctly detected on only 59.8% of trials overall. Using the masking paper 79.5% of trials were correct. The difference in performance in the two conditions is statistically significant ($F=8.2$, $P<0.05$).

It has been established² that the skin contains more than one type of receptor responsive to mechanical deformation. This finding suggests a possible explanation for the effect described. If rubbing an undulating surface commonly induces more than one kind of sensory input, then a highly sensitive system, such as that associated with light pressure, may interfere with the input from deeper pressure receptors, the response to roughness thus masking the response to more gradual surface changes. The effect of placing material under the fingers may be to reduce this masking, thus allowing the appropriate input to be used in detection.

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Gene dosage effect in human trisomy 16

AFTER the discovery of autosomal trisomies in man, the activity of various enzymes in trisomic cells was measured in the belief that proportionality between gene dose and enzyme activity would help in identifying genes carried by trisomic chromosomes^{1–5}. It became apparent, however, that the trisomic state could produce changes in enzyme activity unrelated to gene dose, probably by interfering with normal physiological processes⁶. Therefore, verification of the concept that a simple rapport of proportionality between gene dose and concentration of secondary gene product could exist in cells with autosomal trisomy, as it does in diploid cells with gene mutations or in mouse eggs with different numbers of X chromosomes⁷, had to await the recent advances in human chromosome mapping. We report here the results of a study of gene dose effects in trisomy 16.

Nine trisomic and eleven control cell strains were used to measure the activity of four enzymes: adenine phosphoribosyltransferase (APRT, EC 2.4.2.7), which is coded by a gene on chromosome 16 (ref. 8); hypoxanthine-guanine phosphoribosyltransferase (HGPRT, EC 2.4.2.8) and glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), which are X-linked; adenosine kinase (AK, EC 2.7.1.20), which is coded by an autosomal gene asyntenic with the APRT locus⁹. Information

Table 1 Origin and chromosome complement of cells analysed

Identification number	Tissue of origin	Gestational age*	Foetal size and development	Chromosome complement
RF3112	Amnion	11	6 mm (SD)	47, XY, +16
RF3138	Amnion	16	9 mm	46, XX
RF3308	Amnion	10	3 mm	47, XY, +16
RF3326	Ovary	17	18 cm	46, XX
RF3391	Amnion	17	IES	47, XX, +16
RF3500	Chorion	10	SD	47, XY, +16
RF3592	Amnion	13	4 mm (D)	47, XY, +16
RF3613	Amnion	11	RS + CS	46, XY
RF3633	Testis	20	18 cm	46, XY
RF3663	Amnion	11	2 mm (SD)	47, XX, +16
RF3679	Amnion	16	RES	47, XX, +16
RF3789	Amnion	11	2 mm (SD)	47, XY, +16
RF3802	Amnion	11	2 mm (SD)	46, XX
RF3817	Amnion	11	9 cm	46, XX
RF3877	Amnion	10	1 cm (SD)	46, XX
RF3905	Amnion	11	RES	46, XX
RF3914	Amnion	11	RS + CS	46, XX
RF3958	Amnion	12	IES	47, XY, +16
H9172	Skin	Adult	Adult	46, XY
H10322	Skin	Adult	Adult	46, XY

* Weeks after last menstruation.

IES, Intact empty sac; RS, ruptured sac; CS, cord stump; RES, ruptured empty sac; SD, severely disorganised; D, disorganised.

Cultures were set up by the plasma clot technique and grown in TC199 supplemented with 15% pooled human serum, 2% chick embryo extract and antibiotics (100 IU penicillin G and 100 µg streptomycin ml⁻¹). Stock cultures were kept in liquid nitrogen and recovered for the purpose of this study. 4×10^6 cells of each strain were collected by treatment with 0.075% solution trypsin in phosphate buffered saline (PBS), washed three times in PBS and lysed by freeze-thawing. APRT, HGPRT and AK activities were measured by the procedures of Long *et al.*⁹, G6PD by the method of Giblett¹⁰ and protein by that of Lowry *et al.*¹¹. ¹⁴C-hypoxanthine, ¹⁴C-adenosine and ¹⁴C-adenine were obtained from the Radiochemical Centre, Amersham and ATP, TTP, phosphoribosyl pyrophosphate, NADP and glucose-6-phosphate from Sigma.

on the specimens used in this study is summarised in Table 1.

Assays showing a linear relationship between enzyme activity and extract dilution were used for analysis (Fig. 1).

Enzyme assays (Table 2) showed a 69% increase in APRT activity in trisomic cells. Such an estimate has wide confidence limits and does not differ significantly from the 50% increase expected on the hypothesis of direct and linear proportionality between gene dose and enzyme activity. In contrast, HGPRT, AK and G6PD activities do not differ significantly in trisomic and control cells.

As HGPRT and G6PD are X-linked^{12,13}, we tested whether their activities are influenced by the number of X chromosomes present in the cells—either directly by comparing the levels of

Fig. 1 Graphs showing relationship between cell extract dilution and enzyme activity in a typical experiment. Trisomic (●) and control (○) cell strains were recovered in pairs. When both strains had reached confluency the cells were collected and assayed together to minimise variations caused by the phase of cell growth and the conditions of the assays. Aliquots from the same cell extract were used to assay all the four enzymes and all but G6PD were tested concurrently and in duplicate on three serial dilutions of the cell extract. a, HGPRT; b, APRT; c, AK.

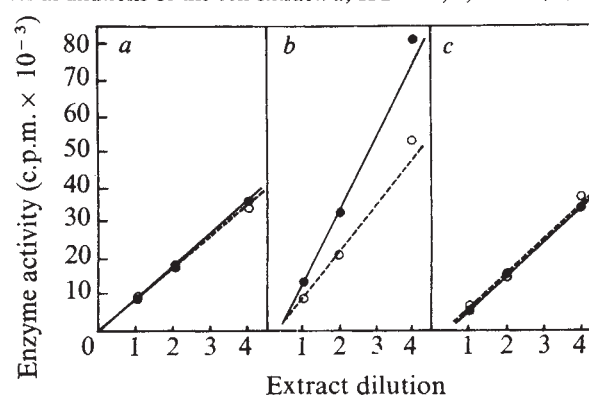


Table 2 APRT, HGPRT, AK and G6PD specific activity in trisomic and control cells

Experimental pair		APRT*		HGPRT†		AK‡		G6PD‡	
Trisomic	Control	Trisomic	Control	Trisomic	Control	Trisomic	Control	Trisomic	Control
RF3391	RF3326	210	122	51	50	—	—	—	—
RF3391	RF3138	150	97	45	48	29	20	0.287	0.286
RF3679	RF3138	151	99	47	50	34.5	20	0.287	0.263
RF3663	RF3138	167	108	43.5	53	20	20	0.345	0.286
RF3500	RF3633	148	72	58	54	26.5	20.5	0.363	0.373
RF3500	RF3613	—	—	—	—	—	—	0.230	0.173
RF3500	H 9172	379	197	47	39	—	—	—	—
RF3500	H10322	242	45	55	40	—	—	—	—
RF3592	RF3633	87	24	38	30	27	24	0.289	0.311
RF3789	RF3633	162	76	39	56	25	20.5	0.251	0.293
RF3391	RF3633	109	76	40	56	29	20.5	—	—
RF3679	RF3633	149	76	44	56	34.5	20.5	—	—
RF3663	RF3633	170	77	46	54	20	20.5	—	—
RF3500	RF3817	53	128	32	65	53	116	0.168	0.182
RF3500	RF3138	133	108	54	53	26.5	20	—	—
RF3789	RF3138	162	108	39	53	25	20	—	—
RF3789	RF3914	109	84	42	57	56	76	0.149	0.183
RF3308	RF3905	45	36	54	57	25	28	0.312	0.283
RF3958	RF3877	24	38	—	49	38	49	0.237	0.277
RF3112	RF3802	73	40	40	30	—	43	0.061	0.096
Mean		143.3	84.8	45.2	50.0	31.2	33.0	0.248	0.250
Difference		58.5§		4.8		1.8		0.002	
s.e.		13.9		2.8		4.9		0.010	
Patient/control ratio		1.69		0.90		0.95		0.99	

* AMP nmol per h per mg protein.

† IMP nmol per h per mg protein.

‡ NADP μ mol per min per mg protein.

§ Significant at 0.001 level.

enzyme activity in male and female trisomic and control strains or indirectly by comparing the differences in enzyme activities of controls and trisomic cells in pairs of equal sex and pairs formed by a male trisomic and a female control (last seven in Table 2). These tests indicate that the activities of these two enzymes do not differ significantly in male and female control or trisomic cell strains (Table 3).

Past experience suggests caution in the interpretation of increased enzyme activity in trisomic cells. Note that changes in enzyme activity unrelated to gene dose have usually been observed in blood cells. The latter are in a terminal state of differentiation and are usually tested immediately after their withdrawal from the abnormal environment of the trisomic subject. In this study, trisomic and control cells were grown for some time *in vitro* in identical conditions and analysed in the same growth phase.

Because changes in enzyme activity for physiological rather than strictly genetical reasons may similarly affect enzymes of related metabolic pathways, APRT activity was compared with that of the metabolically related enzymes, HGPRT and AK. In fact the ratio of HGPRT to APRT activity has already been profitably and extensively used in the measurement of gene dose effects in euploid cells—namely, the detection of heterozygotes for mutations at the HGPRT locus^{14–16}. The genes coding for the enzymes considered in this work are well expressed in interspecific somatic cell hybrids and seem, therefore, rather insensitive to abnormal genetic situations. In view of these considerations we believe that our observed increases in APRT activity in cells with trisomy 16 is the direct expression of gene dose and is not secondary to a change in cell physiology. Our findings, therefore, provide some evidence for a linear and directly proportional relationship between gene dose and enzyme activity.

A dosage effect for the two loci assigned to chromosome 21 has also been reported in Down's syndrome. Sinet *et al.*¹⁷ have observed a 40% increase in the activity of the cytoplasmic form of superoxide dismutase (SOD-1) in erythrocytes of patients with trisomy 21. Unfortunately, however, they have not studied the activity of other enzymes or tested SOD-1 in other types of cells. The value of their observation is, therefore, greatly

reduced by the fact that erythrocytes in Down's syndrome have repeatedly shown increases in the activity of enzymes not coded by chromosome 21. Tan *et al.*¹⁸ have studied the relationship between gene dose and the production of antiviral protein (AVP) in trisomy 21 by measuring the antiviral protection afforded by this protein to trisomic cells. AVP activity is induced by interferon and cells with trisomy 21 have been shown to be three to seven times more responsive to such induction than cells with trisomy 13 or 18, or normal chromosome complement. It is at present unclear whether the nonlinear relationship observed between AVP gene dose and antiviral protection reflects the effect of gene dose on protein synthesis or that of AVP concentration on viral infection. The answer to this dilemma and comparisons between the behaviour of inducible and constitutive enzymes in cells with abnormal karyotypes may help us to understand, in biochemical terms, the phenotypic effect of chromosome imbalance.

Table 3 Sex differences in HGPRT and G6PD activity in trisomic and control cells

	HGPRT activity		G6PD activity	
	Difference	Probability	Difference	Probability
Control cells (♂ against ♀)	-4.3	>0.5	0.063	>0.1
Trisomic cells (♂ against ♀)	0.68	>0.5	-0.077	>0.1
Pairs of equal sex against pairs of unequal sex*	6.2	>0.2	0.028	>0.1

* Only pairs of unequal sex comprising a male trisomic and a female control are considered.

Our HGPRT and G6PD assays seem to support the evidence for dosage compensation of X-linked genes in man by suggesting that compensation takes place even in 8–14-week-old zygotes, whose development has been severely impaired by trisomy 16.

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cross to obtain both genes segregating simultaneously. It is therefore not so astonishing that Mendel did not run into the complication of linkage, although he did not avoid it by choosing one gene from each chromosome.

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Defect of macrophage function in the antibody response to sheep erythrocytes in systemic *Mycobacterium lepraemurium* infection

THE need for macrophages for optimal antibody responses, both *in vivo* and *in vitro*, to certain antigens is now established^{1,2} and this paper describes experiments which have used this requirement to demonstrate a deficiency of macrophage function in mice experimentally infected with the rodent leprosy bacillus, *Mycobacterium lepraemurium*. This organism is an obligate intracellular dweller and is found particularly inside cells of the macrophage series although in terminal infection other cell types may be invaded. Systemically infected mice characteristically show macrophages overloaded with bacilli, ever increasing numbers of granulomata and increasing spleno- and hepatomegaly³. There is an increase in the phagocytic activity of the spleen and liver at an early stage of infection (I.N.B. and V.S.S., in preparation) indicative of alterations in macrophage function and we report here experiments which show that the *in vivo* and *in vitro* antibody response to sheep erythrocytes (SRBC) is depressed at later stages of infection. The results indicate a defect of macrophage function.

Young adult CBA female mice were injected intravenously with 10⁹ *M. lepraemurium* freshly isolated from the heavily infected livers of mice infected 4–6 months previously (I.N.B. and H. N. Krenzien, in preparation). At 11–15 weeks after infection, when the present experiments were carried out, the mice appeared clinically healthy but at autopsy showed gross enlargement of liver and spleen. Ziehl-Neelsen stain revealed the presence of numerous mycobacteria in macrophages of various tissues, particularly liver, spleen and bone marrow. Uninfected mice of the same age and sex were used as controls. The *in vivo* antibody response was measured in mice injected intravenously with either 5 × 10⁶ or 10⁹ SRBC. Four days later, the number of antibody producing cells (PFC) present in the spleen of each mouse was determined using the method of Cunningham⁴. The *in vitro* response of spleen cells cultured for 4 d according to the method of Marbrook⁵ was measured in the same way.

The results given in Table 1 show that the antibody response *in vivo* was reduced 11 weeks after infection and even more so at 14 weeks. This depression was more pronounced in animals injected with the smaller number of SRBC. It seemed greater when the results were expressed as PFC per 10 spleen cells because there was a 4–5-fold increase in the cellularity of the spleens from infected compared with normal mice. Serum antibody levels were also reduced in infected mice (results not shown). Spleen cells from infected animals cultured *in vitro* in the presence of SRBC failed to generate the expected number of PFC, particularly at 14 weeks.

Why didn't Gregor Mendel find linkage?

It is quite often said that Mendel was very fortunate not to run into the complication of linkage during his experiments. He used seven genes and the pea has only seven chromosomes. Some have said that had he taken just one more, he would have had problems. This, however, is a gross oversimplification. The actual situation, most probably, is shown in Table 1. This shows that Mendel worked with three genes in chromosome 4, two genes in chromosome 1, and one gene in each of chromosome 5 and 7. It seems at first glance that, out of the 21 dihybrid combinations Mendel theoretically could have studied, no less than four (that is, *a-i*, *v-fa*, *v-le*, *fa-le*) ought to have resulted in linkages. As found, however, in hundreds of crosses and shown by the genetic map of the pea¹, *a* and *i* in chromosome 1 are so distantly located on the chromosome that no linkage is normally detected. The same is true for *v* or *le* on the one hand, and *fa* on the other, in chromosome 4. This leaves *v-le*, which ought to have shown linkage.

Mendel, however, seems not to have published this particular combination and thus, presumably, never made the appropriate

Table 1 Relationship between modern genetic terminology and character pairs used by Mendel

Character pair used by Mendel	Alleles in modern terminology	Located in chromosome
Seed colour, yellow-green	<i>I-i</i>	1
Seed coat and flowers, coloured-white	<i>A-a</i>	1
Mature pods, smooth expanded-wrinkled	<i>V-v</i>	4
Inflorescences, from leaf axils-umbellate in top of plant	<i>Fa-fa</i>	4
Plant height, > 1m-around 0.5 m	<i>Le-le</i>	4
Unripe pods, green-yellow	<i>Gp-gp</i>	5
Mature seeds, smooth-wrinkled	<i>R-r</i>	7

Table 1 Antibody response of mice, or spleen cells from mice, infected with *M. lepraemurium*

Parameter of response	Dose of SRBC	Control	Weeks after infection	
			11	14
<i>In vivo</i>				
log ₁₀ PFC per spleen	5 × 10 ⁶	5.85 ± 0.02	5.33 ± 0.06	4.87 ± 0.16
	10 ⁹	5.97 ± 0.04	5.76 ± 0.07	5.28 ± 0.03
PFC per spleen, geometric mean (× 10 ⁻³)	5 × 10 ⁶	703	214	75
	10 ⁹	937	570	189
PFC per 10 ⁶ spleen cells	5 × 10 ⁶	6,051 ± 391	605 ± 100	204 ± 83
	10 ⁹	4,899 ± 452	1,250 ± 154	446 ± 21
<i>In vitro</i>				
PFC per culture	—	2,086 ± 142	1,246 ± 57	290 ± 39

Spleen cells were cultured in RPMI 1640 medium containing HEPES, bicarbonate and 5% foetal calf serum. The cultures contained 20 × 10⁶ spleen cells and 2 × 10⁶ SRBC. The values shown are means ± s.e. of four mice in each group or quadruplicate cultures of spleen cells pooled from two or three mice. The significance of difference between control and experimental groups, calculated using the two-tailed Student/Welch's *t*-test⁶, was at the level of *P* < 0.005 or higher.

Further studies were carried out *in vitro* to obtain information about the functional status of macrophages and lymphoid cells in infected spleens. Cells from infected and control mice were fractionated into adherent (macrophage) and non-adherent (lymphocyte) populations and were then cultured in various combinations in the Marbrook system. The results are shown in Table 2. Cultures containing only non-adherent cells produced small numbers of PFC and no PFC were detected in cultures of peritoneal cells or adherent spleen cells (not shown in the table). When non-adherent cells from the spleens of normal mice were supplemented with normal peritoneal cells or adherent

spleens were supplemented with either normal peritoneal cells or normal spleen adherent cells (experiments 2 and 3).

The results presented here indicate, in contrast to another report⁹, that the antibody response to SRBC is depressed in mice systemically infected with *M. lepraemurium*. Furthermore, the defect was associated particularly with the macrophages of infected animals and not with their lymphocytes at the stages of infection studied. Our experiments do not indicate a direct contact effect by heavily infected macrophages or the release of a suppressive factor. The nature of the defect has yet to be established. Two possibilities are being considered in our immediate experi-

Table 2 The *in vitro* antibody response of spleen cells from mice infected with *M. lepraemurium*

Experiment	Cells cultured	Control	PFC per culture	
			Weeks after infection	
			11	14-15
1	Spleen unfractionated	2,140 ± 82	960 ± 57	620 ± 22
2	Spleen non-adherent + normal PC	2,140 ± 85	1,790 ± 78	1,466 ± 33
3	Spleen non-adherent + normal spleen adherent	1,886 ± 41	1,800 ± 26	1,706 ± 63
4	Spleen unfractionated + normal PC	2,600 ± 10	1,716 ± 51	1,510 ± 59
5	Spleen non-adherent	190 ± 12	96 ± 46	80 ± 34
6	Normal spleen non-adherent + infected spleen adherent	—	1,192 ± 64	610 ± 13

Cultures of unfractionated spleen cells contained 20 × 10⁶ cells ml⁻¹. Non-adherent cells were obtained by incubating spleen cells (20 × 10⁶ ml⁻¹) twice for 30 min at 37 °C on glass and the free cells in the suspension were then gently agitated and poured off and 1 ml put into each culture vessel. The adherent spleen cells were removed from the glass surface by means of a rubber policeman before which the surface was vigorously washed with fresh medium. Peritoneal cells (PC) were obtained by washing the peritoneal cavity of untreated mice. Cultures were supplemented with macrophages by adding 2 × 10⁶–3 × 10⁶ viable adherent spleen cells or 4 × 10⁶ peritoneal cells. All cultures contained 2 × 10⁶ SRBC. Values given are means ± s.e. of quadruplicate cultures.

spleen cells (experiments 2 and 3), the PFC response was similar to that found in cultures of unfractionated normal spleen cells. This result confirms earlier findings^{7,8} on the requirement for macrophages in the primary response to SRBC *in vitro*. On the other hand, when adherent cells from the spleens of mice infected 11 or 14–15 weeks before were used to supplement non-adherent normal mouse spleen cells (experiment 6) the number of PFC found in the cultures was similar to that found in cultures of unfractionated spleen from infected animals (experiment 1). This result suggested that macrophages from infected mouse spleens were defective in some respect or, alternatively, that they had an inhibitory effect on the function of lymphocytes. These possibilities were tested by adding normal mouse peritoneal cells to cultures of unfractionated spleen cells from normal or infected animals (experiment 4). Under these conditions, the PFC response of the cultures prepared from infected animals was substantially improved (compare experiments 1 and 4) indicating that the presence of infected macrophages did not greatly interfere with the PFC response provided that adequate numbers of normal macrophages were present.

Further evidence for the essentially normal function of the lymphoid cells of infected mice was obtained from experiments where non-adherent cells from infected mouse

spleens were supplemented with either normal peritoneal cells or normal spleen adherent cells (experiments 2 and 3). First, that overloading of macrophages with mycobacteria may interfere with their ability to ingest, process and present other antigens in a normal manner¹⁰, and second, a possibility associated with the massive antibody production against *M. lepraemurium* antigens during infection, that antigen-antibody complexes are formed and become bound to the surface of macrophages, thus blocking the sites for binding of the factor(s) produced by T lymphocytes which may be required for antibody production to SRBC. Whether or not macrophage defects of a similar nature occur in diseases of man where macrophage involvement is extensive, for example, leprosy, leishmania and malaria, requires further study. If they do, they could account, at least in part, for the immunosuppressed state often associated with such infections.

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Promotion of cell fusion by divalent cation ionophores

THE importance of cytoplasmic calcium has recently been emphasised by demonstrations that the ionophores X537A and A23187, which are known to facilitate the movement of Ca^{2+} through membranes^{1,2}, trigger secretion in the presence of extracellular calcium ions^{3,4}. We now report observations on the effects of these two cation ionophores on hen erythrocytes at physiological pH, which raise the possibility that the phenomenon of cell fusion may also be mediated by an increased concentration of cytoplasmic Ca^{2+} .

A suspension of hen erythrocytes (about 6×10^8 cells ml^{-1}) in buffered solution (A), (NaCl 116 mM, CaCl_2 1 mM, $\text{Me}_2\text{AsO}_2\text{Na}$ 10 mM, glucose 1 g l^{-1} , penicillin G 200,000 units l^{-1} , streptomycin sulphate 100 mg l^{-1} ; pH 7.4) was prepared⁵. After centrifugation, 1 ml of the packed cells was incubated with 250 units of neuraminidase solution (*Vibrio cholerae*) (0.5 ml; Behringwerke AG) for 30 min at 37 °C. The treated cells were then washed twice with the buffered salt solution (10 ml), diluted to give a cell suspension of $\sim 6 \times 10^8$ cells ml^{-1} , and kept at 4 °C until used later on the day of preparation.

Suspensions of neuraminidase-treated cells ($\sim 2 \times 10^8$ cells ml^{-1}) in buffered salt solution (A) containing dextran (80 mg ml^{-1} ; MW 82,000; Sigma Chemical Co.), were incubated at 37 °C with either X537A or A23187 (6.6 μg ml^{-1}). X537A and A23187 were given by Roche Products and Eli Lilly respectively. The ionophores were dissolved in ethanol (1 mg ml^{-1}). Small aliquots of cell suspension were removed at intervals for microscopy. In control experiments, neuraminidase-treated cells were incubated with ethanol (6.6 μl ml^{-1}) in the dextran-containing medium, free from ionophore.

After treatment with neuraminidase, the erythrocytes

aggregated into large clumps on incubation at 37 °C with dextran, and the outlines of individual cells in these clumps could not easily be distinguished (Fig. 1a). A similar dextran-induced aggregation has been reported to occur with neuraminidase-treated human erythrocytes⁶. When ionophore was also present, individual cells in the clumps became rounded and swollen within 15 min. The nuclei were clearly visible in these preparations and limited cell fusion may have occurred after 3 h (Fig. 1b). A further incubation of the cell suspensions at 47 °C for 10 min resulted in extensive fusion (Fig. 1d). Essentially similar results were obtained with both ionophores. Without ionophore, the neuraminidase-treated, dextran-aggregated cells were apparently unaffected after 30 min further incubation at 47 °C (Fig. 1e). Electron microscopy confirmed cell fusion in the cells treated with ionophore (Fig. 1c), but no fusion was seen in its absence. Only a limited degree of cell fusion occurred, even at 47 °C, when the concentrations of ionophores were reduced tenfold (0.66 μg ml^{-1}).

A first requirement for cell fusion is cell contact. In the presence of lysolecithin hen erythrocytes aggregate and fuse at pH 5.6 (ref. 7). With fusogenic lipids, for example glyceryl mono-oleate, fusion at pH 5.6 is facilitated by dextran which increases the aggregation of hen erythrocytes treated with the fusogen⁸. Following neuraminidase action, the cells are more rapidly fused even at pH 7.4 by glyceryl mono-oleate, in the presence of dextran⁸. Divalent cation ionophores seem to have little aggregating action and, for fusion to occur rapidly, it seems to be necessary to treat the cells with neuraminidase and then to aggregate them with dextran. Cell fusion occurred, however, in the presence of 2 mM Ca^{2+} , even at 37 °C, with hen erythrocytes that were not pretreated with neuraminidase but were incubated in dextran-containing media for 5 h with A23187 or 18-22 h with X537A.

Membrane fluidity is also important in cell fusion^{9,10}, and changes in membrane structure resulting from treatment with X537A and A23187 that lead to cell fusion are markedly accelerated by raising the temperature to 47 °C. (Thermally induced cell fusion at 48-50 °C and pH 5.6 in the absence of ionophore, which was reported earlier⁸, occurs only on a heated, microscope stage and not with cells in free suspension.) Alternatively benzyl alcohol (30 mM), which increases membrane fluidity¹⁰ but is not fusogenic at this concentration¹¹, may be used instead of heat to aid the induction of cell fusion by ionophore. Mechanical tapping of a microscope slide

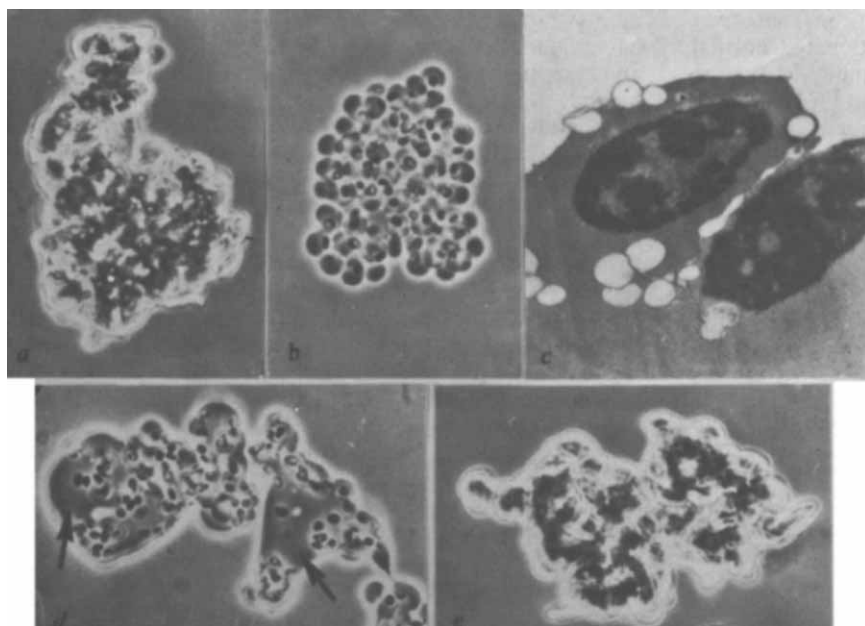


Fig. 1 a, b, d and e, Light micrographs (phase contrast, $\times 300$) of neuraminidase-treated hen erythrocytes at pH 7.4 prepared as described in the text. a, A clump of aggregated cells formed on incubation at 37 °C for 5.75 h with dextran (80 mg ml^{-1}) and ethanol (6.6 μl ml^{-1}). b, Swollen, aggregated cells arising on incubation at 37 °C for 3 h with dextran (80 mg ml^{-1}), ethanol (6.6 μl ml^{-1}), and X537A (6.6 μg ml^{-1}). d, Cells treated as in b followed by incubation at 47 °C for 10 min: multinucleated syncytia are present that contain aggregated nuclei, and areas of nuclei-free cytoplasm (arrows). e, Cells treated as in b, but without ionophore, followed by incubation at 47 °C for 30 min. c, An electron micrograph of a thin section, prepared as described previously⁷, of cells that were incubated at 37 °C for 1 h with dextran (80 mg ml^{-1}), ethanol (6.6 μl ml^{-1}), and A23187 (6.6 μg ml^{-1}), followed by 45 min at 47 °C, showing two nuclei in a common cytoplasm: vacuolation of the plasma membrane is also apparent ($\times 8,250$).

bearing a preparation like that shown in Fig. 1b will also trigger some of these cells to fuse.

Light microscope experiments provided no evidence that either valinomycin ($6.6 \mu\text{g ml}^{-1}$), with or without 2,4-dinitrophenol (0.1 mM), or 2,4-dinitrophenol alone caused the erythrocytes to fuse. Increasing the concentration of Ca^{2+} from 1 to 3 mM allowed the changes, including cell fusion, caused by X537A and A23187 to occur more rapidly, but was without effect on cells treated with valinomycin or valinomycin and DNP. The actions of X537A and A23187 with 1 mM Ca^{2+} were inhibited by EGTA and EDTA (2 mM); inhibition by the chelating agents was overcome by excess Ca^{2+} (final concentration 3 mM). It may therefore be concluded—as with secretory systems^{3,4}—that the observed fusogenic action of these two divalent ionophores is mediated by entry of Ca^{2+} into the cells.

Ca^{2+} probably facilitates cellular aggregation in cell fusion. On the basis of our experiments it is also conceivable that Ca^{2+} may mediate cell fusion, occurring either naturally or in response to fusogenic agents⁵, by interacting with the cytoplasmic side of the plasma membrane following an initial increase in membrane permeability to exogenous Ca^{2+} . This would apparently be contrary to the views of Poste and Allison¹² who have postulated that membranes can exist in two states, (1) the normal Ca^{2+} -associated state; and (2) the “fusion susceptible” state in which Ca^{2+} has been displaced from the membrane.

Cell swelling, which accompanies fusion induced by ionophores and lipid fusogens⁵, may be a result of inhibition by cytoplasmic Ca^{2+} of membrane-bound Na^+/K^+ -dependent ATPase activity, with a resultant loss of cytoplasmic potassium¹³, and the entry of external water as postulated for the Ca^{2+} -dependence of the acrosome reaction¹⁴. Ca^{2+} may also be expected to aggregate negatively charged spectrin molecules on the cytoplasmic surface: this will, in turn aggregate integral membrane proteins¹⁵. Furthermore the binding of Ca^{2+} to phosphatidylserine molecules in a membrane yields solid aggregates that allow other phospholipids to form fluid clusters¹⁶. If this should occur at the cytoplasmic surface of the erythrocytes, where most of the phosphatidylserine is located¹⁷, it would probably also lead to an aggregation of intramembranous proteins¹⁸. Aggregation of membrane proteins^{12,19} and the clustering of lipid molecules¹⁹ are thought to be important in membrane fusion. These phenomena may therefore all participate in cell fusion induced by an increase in the concentration of cytoplasmic Ca^{2+} . Some of these suggestions may also be relevant to the Ca^{2+} -mediated fusion of membranes that occurs in secretory processes^{20,21}.

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Differential formation of desmosomes and hemidesmosomes in epidermal cell cultures treated with retinoic acid

In various epithelial tissues, desmosomes (D) as well as hemidesmosomes (HD) are common specialisations of the plasma membrane¹. Both membrane differentiations are thought to play an important role either in cell–cell adhesion (D) or in the attachment to biological substrata (HD)². *In vitro* formation of D and HD has been observed in embryonic lens cells³ or organ culture outgrowth of skin⁴. We have now found that in cultured post-embryonic epidermal cells (PEC) both membrane specialisations are formed independently of each other. We show that in cells stimulated to grow, an increased number of attachment devices contacting the substratum is formed. At the dorsal surface of the same cell, however, morphological expression of tissue-specific differentiation is present.

In cultured PEC (ref. 5), nuclear DNA synthesis as well as cellular growth is greatly enhanced by the addition of retinoic acid (RA) to the culture medium. Comparison with other cell systems present in skin indicates tissue specificity⁶. The mitogenic effect of RA was therefore used to study the formation of membrane specifications under different growth conditions.

Figure 1 shows that numerous specialisations of the plasma membrane are present in 3-d-old cultures. HD are exclusively present at the underside of ‘basal’ cells contacting the plastic substratum. On the other hand, D are located at the upper side of ‘basal’ cells facing overlying epidermal cells. The morphology of both, HD and D, does not seem to be different from previous *in vivo* descriptions^{1,2}.

Twice as many HD were present at the undersurface of RA-treated cells by comparison with controls ($P < 0.005$, Fig. 2). Functionally, this greater number of HD present in treated cells should indicate increased adherence to the substratum. Considerably longer exposure times to trypsin are necessary, however, to collect RA-treated cells as compared with untreated controls (E. C. and H. H. W., unpublished).

The number of D at the dorsal site of the ‘basal cell’ plasma membrane contacting the suprabasal cell did not change with treatment (Fig. 2). Obviously, within the same cell the site-dependent attachment devices are formed independently.

In epithelial cells freshly treated with trypsin, D and HD are absent³, and are therefore most likely to be newly formed during cellular reaggregation. Also, formation of D depends on the presence of adjacent cells of the same species and of a corresponding maturational age³. Our observed distribution of both membrane specialisations follows the *in vivo* pattern of epidermal differentiation.

In contrast to tumour cells, so-called ‘normal’ cells show strong anchorage dependence when grown *in vitro*⁷. It has been found⁸ that changing the substratum (from glass to agarose) under otherwise identical conditions resulted in

decreased adherence of cells, reduced rate of DNA synthesis and increased generation time. It thus seems that in normal cells attachment to the substratum is linked to cellular proliferation.

There is evidence that in epidermal cells such fixation to the substratum is provided by hemidesmosomes. Our observation of a greatly increased number of HD in rapidly multiplying cells supports the concept that these membrane specialisations are positively correlated with cell proliferation. Under *in vivo* conditions strong adherence to the basal

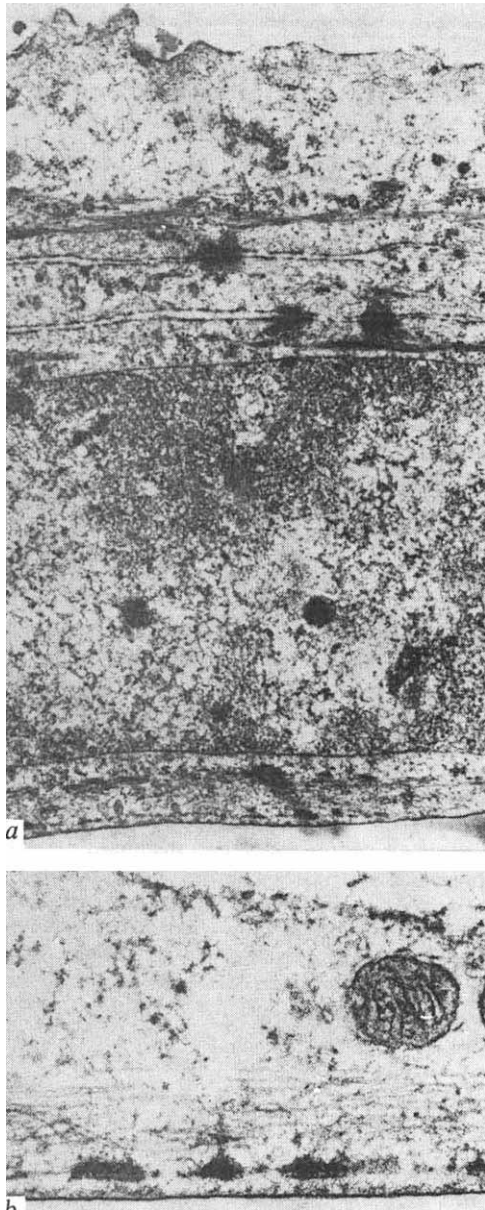


Fig. 1 High density epidermal cell culture grown *in vitro* for 3 d (control). Two types of membrane specialisations are formed: D, located at sites in contact with adjacent cells (a), and HD, exclusively present at the undersurface of cells contacting the substratum (b). Cultures were prepared³ by gentle treatment with trypsin of adult guinea pig ear skin fragments. Cell suspensions consisting of single cells were seeded at a density of $1.8\text{--}2.2 \times 10^6 \text{ ml}^{-1}$ in 35 mm Falcon culture dishes and maintained in 5% CO_2 -air at 37°C . *trans*-Retinoic acid (Nordmark-Werke, Uetersen) was dissolved in DMSO and added to the culture medium (McCoy 5a, supplemented with 10% foetal calf serum, penicillin and streptomycin) at a final concentration of $10 \mu\text{g ml}^{-1}$. The concentration of DMSO in all cultures, including controls, was 0.1%. a, $\times 27,540$; b, $\times 60,000$.

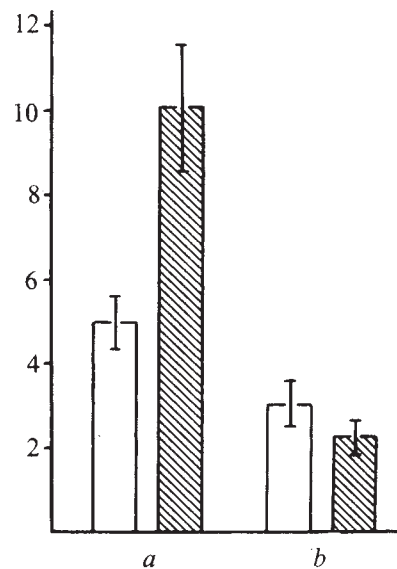


Fig. 2 Number of HD (a) and D (b) in 3-d-old cultures of PEC treated with $10 \mu\text{g ml}^{-1}$ RA (hatched bars) and untreated controls (open bars). Epon-embedded specimens were obtained after *in situ* fixation of entire culture plates. Selected areas were excised and cut with a diamond knife. Vertical sections were stained with uranyl acetate and lead citrate, and viewed with a Philips EM300 microscope. Photomicrographs were taken for HD and D counts at magnifications between 8,000 and 26,000. Length of plasma membrane on both sides of the cells was measured using a map roller. HD and D were counted and the results calculated as HD or D per $10 \mu\text{m}$ surface length $\pm$ s.e.

lamina would thus be provided for cells engaged in reduplication.

On the other hand, upward movement and tissue-specific differentiation of epidermal cells coincides with the disappearance of hemidesmosomes and increased formation of desmosomes. The basic functional difference between the two attachment devices of the epidermal cell membrane thus seems to be closely linked to the mechanism of epidermal cell renewal.

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Demonstration of intermolecular forces in cell adhesion using a new electrochemical technique

THE problem of cell adhesion has been approached from both chemical¹⁻³ and physical^{4,5} viewpoints. We present here new evidence that a purely physical attraction acts between cells and other surfaces and that electrostatic forces can overcome this attraction. Previous attempts to demonstrate

and interpret such forces⁶⁻⁸ have largely been frustrated by inadequately defined test surfaces, biological media including complex macromolecular components, and inappropriate assays for cell adhesion.

The unique features of our experimental approach are that the interfacial electrostatic charge of a well defined clean metallic surface can be varied continuously from positive to high negative values, and measured accurately. We have consequently been able to determine the critical electrostatic condition for cell adhesion. Another significant feature of our system is that the very low contamination rate of the test surface can be monitored continually during the course of the experiment.

We have used the relatively new development of solid polarisable electrodes: the test surface was a lead electrode⁹ whose high polarisability in contact with electrolyte enables the interface to be charged positively or negatively by means of a battery and potentiometer: the magnitude of the charge being determined accurately from differential capacitance measurements. Since a current density of only a few $\mu\text{A cm}^{-2}$ flowed across the electrode surface, the experiment was virtually electrostatic. The lead surface was prepared by chemical polishing, after which it is probably molecularly smooth over large areas. Contamination of the electrode by oxygen, the adsorption of ions or organic molecules, could be detected by capacitance changes, providing a criterion for the rejection of poorly prepared electrodes. In practice, high stability over a 6-h period was frequently achieved following exhaustive cleaning and deoxygenation of the entire system.

We examined the adhesion of glutaraldehyde-fixed human red blood cells to the electrode in dilute sodium fluoride solutions of electrochemical purity. Aldehyde fixation, which has little or no effect on cell surface charge¹⁰, was necessary to render cells stable at low ionic strength and to avoid loss of cellular proteins which might otherwise contaminate the electrode. Adhesion was assessed by allowing cells to settle for twenty minutes (settling for a period of 1 h does not affect the results) on to the polarised electrode and then inverting it while under continuous microscopic observation: cells which fell off were scored as non-adhesive. Cell falling was usually completed in 10 min.

In 10 mM NaF all cells stuck to the electrode, even at high negative surface charge. As the concentration was reduced to 1.1 mM, however, a most interesting pattern of behaviour was seen. When the electrode was positively charged ($+1.2 \times 10^3$ e.s.u. cm^{-2}) the negatively charged cells adhered irreversibly—our criterion for irreversibility being that the cells continued to adhere when the negative polarisation was subsequently increased to the maximum value of -4.1×10^4 e.s.u. cm^{-2} , approximately ten times the cell surface charge density. But when cells were allowed to settle on the negatively polarised electrode, within the range -5×10^3 to -3×10^4 e.s.u. cm^{-2} , reversible adhesion occurred. The cells failed to fall off when the electrode was inverted even after 20 min, but within a few minutes of the negative polarisation being increased beyond -3×10^4 e.s.u. cm^{-2} most of the cells were forced off electrostatically and were seen to sediment away from the electrode under the effect of gravity. Cells which adhered to the electrode at different charge densities within the range -5×10^3 to near -3×10^4 e.s.u. cm^{-2} fell off close to the critical charge density of -3×10^4 e.s.u. cm^{-2} .

The explanation for these entirely novel observations can be provided in terms of electrostatic and electrodynamic forces. The physical force theory² predicts strong, probably irreversible, adhesion at the limit of molecular approach (primary potential energy minimum) and a weaker reversible adhesion where cell and surface are separated by a finite gap and the forces of attraction and repulsion are equal (secondary potential energy minimum), the two energy minima being separated by an energy barrier.

Preliminary calculations suggest that the distance of closest approach between a red cell and the metal surface may be several hundred Å in the secondary minimum position. We therefore feel that the essential criteria for the duplex adhesive behaviour predicted by the physical force theory have been demonstrated, and it is hard to think of an alternative explanation for the experimental facts. It is anticipated that when the distance of separation between cell and electrode has been measured directly it will be possible to estimate the size of the attractive force from the repulsion and make a quantitative comparison with the theory.

Whether secondary minimum adhesion occurs in physiological conditions remains uncertain. In our experimental system it has been found only at very low ionic strength where electrostatic repulsion can be maximised. But the calculated electrodynamic attraction to metal is large³, so that lower repulsion coupled with lower attraction may give reversible secondary minimum adhesions in physiological media. Further experiments on cell adhesion at charged oil-water interfaces¹¹ may answer this question.

Our experiment has demonstrated that attractive forces which presumably include both image forces and electrodynamic (van der Waals') forces, and repulsive electrostatic forces, all of which can act over distances greatly in excess of chemical bond lengths, are responsible for the adhesive behaviour of red blood cells to a defined surface in the most stringently controlled conditions yet used in such work. The very nature of these rigorous controls makes the experiment unavoidably non-physiological; this, however, by no means detracts from the fact that long range interactions have been shown to occur between the complex architecture of protein, lipids and glycoprotein comprising the cell periphery, and the atoms of a solid surface. The conclusion that these forces also operate between cells would seem to be unavoidable.

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Increased spontaneous transmitter release from presynaptic nerve terminal by methylmercuric chloride

IN recent years, several incidences of methylmercury pollution have been documented^{1,2}, one of the most striking alterations induced by the compound being extensive damage to the nervous system. Experimental mercury poisoning has been produced in animals^{3,5}, detailed morphological studies of which have shown that acute changes occur initially in the peripheral nerve fibres and thereafter in the central nerve cells^{3,4}. Little

is known, however, about functional changes in the nervous system, particularly in the initial stages of poisoning. This report describes one possible target site—the synaptic transmission in the sympathetic ganglia—for methylmercury poisoning. The study is also of interest, as a number of heavy metallic ions such as La^{3+} , Co^{2+} and Mn^{2+} have recently been reported to have profound effects on neuromuscular transmission⁶⁻⁹.

solution. Table 1 summarises the results together with those from untreated control animals. Values for the resting potentials and amplitudes of the action potentials were within the control ranges. There was no sign of impairment of impulse transmission from the preganglionic nerve to ganglion cells (Fig. 1a and b). Amplitudes of the spontaneous miniature excitatory postsynaptic potentials (MEPSPs) were also unaffected, but frequencies were increased significantly (Fig. 1c and d), their

Table 1 Electrical activities of the sympathetic ganglion cells before (control) and after a single intraperitoneal injection of CH_3HgCl (1 mg per animal)

	Resting potential (mV)	Action potential† (mV)	Amplitude mEPSP (mV)	Frequency (s ⁻¹)	Conduction velocity‡ (m s ⁻¹)
Control	51 ± 5 (43)	62 ± 8 (43)	1.4 ± 0.6 (25)	0.11 ± 0.09 (25)	1.5 ± 0.6 (25)
After 30 min	54 ± 7 (14)	65 ± 11 (14)	1.3 ± 0.3 (12)	0.56* ± 1.01 (12)	1.8 ± 0.6 (14)
After 1 d	50 ± 5 (10)	60 ± 9 (10)	1.6 ± 0.5 (10)	0.28* ± 0.31 (10)	1.4 ± 0.5 (11)
After 5 d	52 ± 4 (13)	61 ± 6 (13)	1.6 ± 0.5 (12)	0.26* ± 0.27 (12)	1.3 ± 0.4 (12)
After 12 d	51 ± 3 (27)	60 ± 6 (23)	1.6 ± 0.4 (22)	0.28 ± 0.56 (22)	1.3 ± 0.6 (19)
After 30 d	52 ± 5 (9)	60 ± 7 (8)	1.6 ± 0.4 (13)	0.10 ± 0.09 (13)	1.6 ± 0.9 (14)

Values are means ± s.d. Numbers in parentheses indicate number of cells analysed.

*Statistical significance in the difference from control ($P < 0.05$).

†Amplitudes of the action potentials evoked by the preganglionic nerve stimulation.

‡Velocities of the most rapidly conducting impulses in the preganglionic nerve, approximated by an estimation from latency of the first EPSP and distances between stimulating and recording electrodes.

Superior cervical ganglia with the preganglionic nerve fibres isolated from guinea pigs (160–240 g) were used. Preparations were mounted in a constant temperature bath (34–36 °C) perfused with a modified Krebs solution, as described previously¹⁰. A single microelectrode filled with 0.6 M K_2SO_4 was used to record intracellularly the membrane potential of individual ganglion cells. The preganglionic nerve fibres were stimulated with twin platinum electrodes to evoke orthodromic responses in ganglion cells.

In the first series of experiments, each guinea pig was given 1 mg methylmercuric chloride (CH_3HgCl) intraperitoneally in aqueous solution. Inspection of the animals at any time after injection revealed no neurological symptoms of the mercury poisoning, such as an ataxic gait or a crossing phenomenon in the hind limbs, reported to occur in rats when total doses of the administration amounted to more than 10 mg (ref. 3). At 30 min, 1, 5, 12 and 30 d after injection, ganglia were isolated and measurements of the electrical activities made in normal

mean values within 5 d of injection being 240–500% greater than control. After 12 d, there was a similar increase in the mean value, but no statistical significance. This is probably caused by a large variability in values for individual ganglion cells. After 30 d, the increased frequency returned to the control level.

In patients suffering from effects of Niigata methylmercury pollution in Japan, Kanbayashi *et al.*¹¹ have found a significantly reduced value for the velocity of the most rapidly conducted impulse in the motor nerves. On the other hand, Von Burg and Rustam¹² have found negative results in the patients of an outbreak in Iraq. In agreement with the latter, our study shows no significant change in the velocity of the preganglionic nerve (Table 1).

Thus, the first series of experiments shows that one of the earliest manifestations of CH_3HgCl poisoning with small doses could be detectable as an increase in spontaneous transmitter release from the preganglionic nerve terminal, and that the animal recuperates within a relatively short time after such poisoning.

In the second series of experiments, isolated ganglia from intact animals were exposed continuously to a solution of CH_3HgCl (0.04 mM; 10^{-5} g ml⁻¹). Fig. 2a, recorded in normal solution, shows a typical MEPSP occurring at a frequency of 0.13 s⁻¹. Figure 2b, recorded 10 min after exposure to the test solution, shows a sudden appearance of unusually large spontaneous potentials, the largest reaching 13.6 mV at an increased frequency of 4.40 s⁻¹. In three cells tested, average amplitudes (± s.d.) were increased from 0.6 ± 0.1, 1.3 ± 0.6 and 1.2 ± 0.5 mV in normal solution to 7.1 ± 4.3, 7.6 ± 4.8 and 3.3 ± 1.0 mV after exposure, respectively. We cannot readily explain, however, the appearance of the large MEPSPs. An anticholinesterase-like effect of CH_3HgCl can be ruled out, because the time course of the large MEPSPs was not prolonged. One possibility is that the compound may depolarise the preganglionic nerve terminals and trigger action potentials leading to release of large amounts of transmitter. At 15 min after exposure (Fig. 2c), the frequency of MEPSPs was further increased (in the three cells tested, 0.13, 0.13 and 0.12 s⁻¹, in normal solution, to 18.89, 21.20 and 25.60 s⁻¹ after exposure, respectively). Some action potentials were initiated from the MEPSPs (Fig. 2c). Amplitude and configuration of the action potentials were similar to those evoked in normal solution by preganglionic nerve stimulation, indicating little effect of the compound on the postsynaptic cell membrane. At 20 min after exposure (Fig. 2d), the frequency gradually decreased.

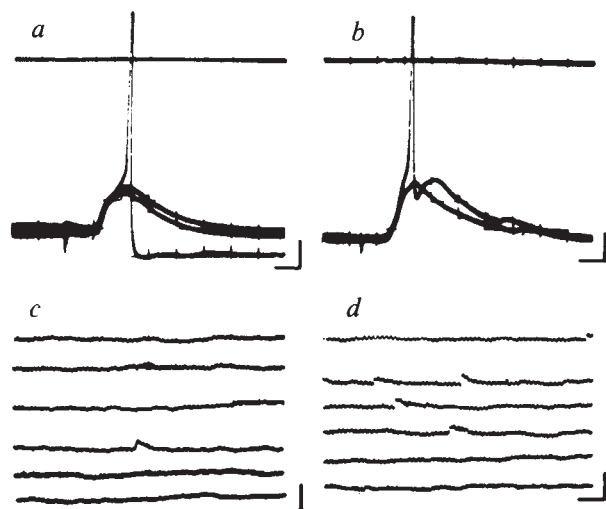


Fig. 1 a, Typical record of action potential and EPSPs in response to three successive stimulations of preganglionic nerve from an intact guinea pig. b, As a, but killed 30 min after intraperitoneal injection of 1 mg CH_3HgCl . c, Typical record (continuous from top to bottom) of MEPSP from intact guinea pig. d, As c, but 30 min after injection. Note increase in frequency of MEPSP in record d. Calibration, 10 mV and 10 ms for records a and b; 5 mV and 0.1 s for records c and d. Records retouched.

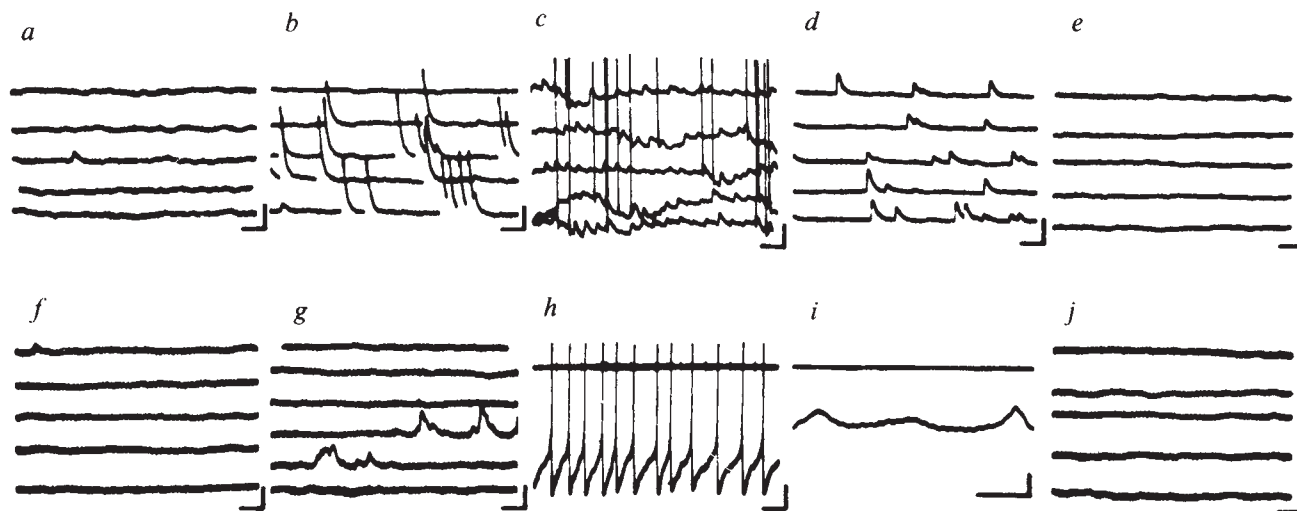


Fig. 2 *a-e*, Application of CH_3HgCl , 0.04 mM, to a ganglion cell by perfusion. *a*, Normal solution; *b-e*, 10 min, 15 min, 20 min and 40 min after exposure. *f-j* HgCl_2 , 0.04 mM. *f*, Normal solution; *g-j*, 30 min, 45 min, 80 min and 90 min after exposure. All records, except *h* and *i*, continuous from top to bottom. *h* and *i*, upper straight line shows zero potential. Calibration, 5 mV and 0.1 s for *a*, *b*, *e*, *f*, *g* and *j*; 10 mV and 0.1 s for *c*, *d*, *h* and *i*. See text for explanation. Records retouched.

At 40 min after exposure (Fig. 2*e*). MEPSPs ceased. At that time, the preganglionic nerve stimulation could not evoke any response in the ganglion cell, indicating that progress of the impulse to nerve terminals was blocked, and/or the transmitter substance was completely exhausted by the compound.

For comparison, similar experiments were carried out with an inorganic mercury compound HgCl_2 (0.04 mM; 1.34×10^{-5} g ml $^{-1}$) (Fig. 2*f-j*). HgCl_2 also caused increases in amplitude and frequency of the MEPSPs (Fig. 2*g*). Compared with CH_3HgCl , however, the increases were smaller and appeared after much longer exposure (more than 25 min). In three cells tested, amplitudes were increased from 1.5 ± 0.5 to 2.6 ± 1.2 , 1.5 ± 0.9 to 3.4 ± 2.3 and 1.7 ± 0.4 to 2.0 ± 0.8 mV, and the frequencies from 0.28 to 6.14, 0.07 to 2.38 and 0.36 to 0.78 s $^{-1}$ after exposure. At 45 min after exposure (Fig. 2*h*), the cell was gradually depolarised and underwent repetitive firing for more than 10 min. Further exposure (Fig. 2*i*) resulted in sustained depolarisation of the membrane to about 30 mV, and the firing ceased leaving only a local fluctuation (similar to a response) in the membrane potential. Eventually, 90 min after exposure, the cell became electrically silent (Fig. 2*j*).

Thus, it has been shown in *in vitro* experiments that the primary site of CH_3HgCl action is on the presynaptic nerve terminal, from which rapid and large spontaneous transmitter release occurs. HgCl_2 actions are both presynaptic, resulting in the release of relatively small quantities of transmitter, and postsynaptic, resulting in the sustained depolarisation of ganglion cells. In conclusion, mercury compounds, particularly CH_3HgCl , resemble the La^{3+} ion which is reported to cause also a rapid and large increase in the frequency of miniature endplate potentials in frogs⁶.

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Chemotactic stimulation by cell surface immune reactions

CHEMOTAXIS of migratory leukocytes is of special interest to immunologists as it is intimately associated with tissue reactions initiated by immune responses. Chemotactic activity as measured *in vitro* with the Boyden chamber has been exclusively associated with chemotactic factors of various origins which all have in common the inherent ability to attract the responding cells. Immune-related chemotactic factors attracting neutrophils (polymorphonuclear leukocytes, PMNs) and macrophages are either complement (C)-derived as a result of C activation or they are products of activated lymphocytes. Thus, their activity is secondary to a specific humoral or cellular immune reaction taking place independent of and at a distance from the responding cell. Here we present a new concept of chemoattraction in which the chemotactic stimulus is a direct result of an immune reaction that proceeds on the surface of the responding cell itself. This concept originated from the discovery of the chemotactic stimulation of PMNs by cytotoxic sera at subcytotoxic doses.

This phenomenon was first observed in heterologous systems, nurse shark and dog sera cytotoxic for human PMNs (refs 1 and 2), and then for homologous systems, human and dog anti-sera against histocompatibility antigens that were cytotoxic for human and dog PMNs, respectively, (J. A. J., and V. E., unpublished). The conditions in which chemotaxis was observed in these cytotoxic systems are shown in Fig. 1*a*, *b* and *c*. We interpreted these results to mean that cytotoxic immune reactions can cause an 'irritation' at the cell surface acting as a chemotactic stimulus provided at least one of the necessary reactants (antibody or the C system or even one limiting C component) is presented to the responding cell on a positive concentration gradient. In the following we show that cells carrying

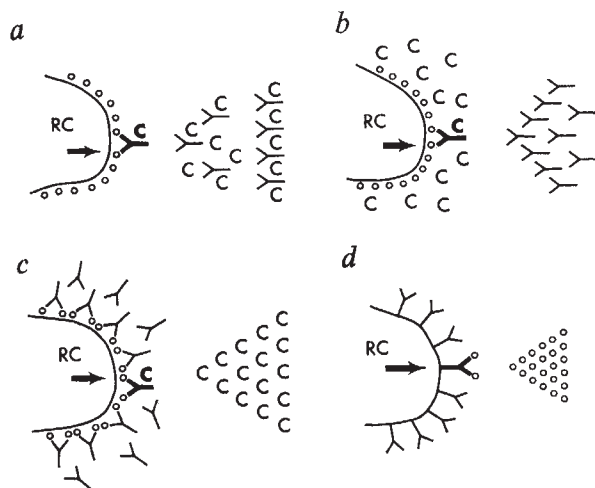


Fig. 1 Schematic representation of different modes of chemotactic stimulation by cell surface immune reactions. RC, Chemotactically responding cell (PMN, macrophage or lymphocyte); O, antigen; Y, antibody or recognition molecule; C, complement or limiting complement component; arrow, direction of chemotactic migration; symbols arranged as wedges, corresponding concentration gradient; symbols on cell surface, stimulating complex. *a-c*, Cytotoxic situations; *d*, antibody is cytophilic or, hypothetically, recognition antibody and the chemotactic gradient is formed by the antigen.

cytophilic antibody can be chemoattracted by the specific antigen. This situation could be of great biological significance, and is illustrated in Fig. 1*d*.

English short hair female guinea pigs (600 g) were immunised intracutaneously at three sites with a total of 3 mg crystalline ovalbumin in Freund's complete adjuvant. Two to three weeks later they were injected intraperitoneally with 30 ml 0.1% shell-fish glycogen in saline. After 4 h the animals were bled, killed and their peritoneal PMNs collected in heparinised saline. The cells were washed and, without further preparation, used in the chemotactic assay.

The cell preparations were usually 98% pure. The upper chamber compartment received 2×10^6 PMNs in Gey's medium.

From Table 1 the following conclusions may be drawn: PMNs from immunised animals were chemotactically attracted by the antigen, whereas those from controls were not. PMNs from normal, non-immunised animals, if treated with immune serum, behaved like cells obtained from immunised animals; if treated with preimmune serum they behaved like normal cells. Antigen introduced into the upper compartment of the chamber together with the cells did not increase random movement that could be mistaken for chemotaxis (R. Snyderman, personal communication). The chemotactic response was dependent on the antigen concentration (dose-response). Chemoattraction by the antigen could only be clearly demonstrated if normal guinea pig serum was present at low concentrations in the upper compartment (or in both compartments) of the chemotaxis chamber. Adding the serum to the upper compartment only had two major advantages: if the serum contained any chemotactic factors they would form a negative concentration gradient for the cells; and possible interactions of serum constituents with the chemotactic antigen were minimised.

We are not yet sure whether the requirement for serum in the medium represents C participation in the given circumstances. This possibility is under investigation. As regards the nature of the responsible antibodies, we have preliminary evidence that the responding cells, obtained from immunised animals or from normal animals and treated with immune serum, carry easily detectable antibody on their surface, whereas controls do not. We are probably dealing with cytophilic (homocytotropic) antibodies. It should be mentioned that the chemotactic response was antigen-specific, that is, that an unrelated antigen (bovine serum albumin, BSA) did not chemoattract the cells carrying anti-ovalbumin antibodies.

In considering the mechanism of this kind of chemotaxis, it is tempting to compare it with the phenomenon of hista-

Table 1 Chemotactic stimulation of guinea pig peritoneal immune PMN by the immunising antigen, ovalbumin

PMN (source, treatment)	20	40	CHP at antigen concentrations ($\mu\text{g}/1.7 \text{ ml}$)					600	No antigen
			60	100	200	400			
IGP No. 17	3	7	34	45	102	102	68	18	
IGP No. 22	72	209	184	457	267	164	—	79	
IGP No. 22*	—	—	—	0	0	0	0	0	
NGP No. 28	—	—	—	0	0	23	18	53	
NGP No. 30	0	0	0	0	0	0	0	0	
NGP No. 30, treated with No. 17 serum (1:10)	0	0	0	0	0	0	12	0	
NGP No. 31	—	—	—	0	0	23	18	86	
NGP No. 31, treated with No. 17 serum (1:5)	—	—	—	77	112	197	105	79	
NGP No. 32	—	—	—	8	4	18	33	58	
NGP No. 32, treated with No. 17 serum (1:5)	—	—	—	56	102	269	83	39	
			Distance travelled (μm) at antigen concentrations ($\mu\text{g}/1.7 \text{ ml}$)						
IGP No. 27	—	62	82	93	97	—	103	61	
IGP No. 27, with antigen in upper compartment only	—	—	—	76	—	66	70	61	
NGP No. 33	—	—	—	58	57	45	—	52	
NGP No. 33, treated with pooled immune serum (1:5)	—	—	—	71	69	70	68	52	
NGP No. 33, with antigen in upper compartment only	—	—	—	56	56	57	—	62	

IGP, immunised guinea pig; NGP, normal (non-immunised) guinea pig; CHP, cells per high power field. *In vitro* chemotaxis was carried out and quantified as described⁸. Modified Boyden chambers were set up in triplicate. Results are expressed as average number PMN per high power field on lower surface of Millipore filters (5 μm pore size) separating the upper from the lower compartments of the chambers. Gey's medium was used throughout. Incubation time 3 h, temperature 37 °C in water saturated 5% CO₂ in air. Cells from non-immunised animals were divided and one half was treated for 45 min at 4 °C with homologous immune serum at the indicated final dilution in Gey's medium; the other half was treated in the same way with the corresponding preimmune serum. All cells were thoroughly washed in Gey's medium before they were placed in the upper compartments. Negative controls contained no antigen in the lower compartments. Chemotactic responsiveness of the cells was ascertained for each experiment. In all experiments except that marked by * the upper compartments contained 0.5% normal autologous guinea pig serum. The last five experiments (lower part of table) were quantified by measuring the distance (in μm) that the migrating cells had travelled into the filter⁴.

mine release or degranulation exhibited by mast cells on contact of antigen with their cytophilic antibodies. The same kind of signal that induces this dramatic effect could well cause a migratory cell to move in the direction of its stimulated surface area.

We believe that this new concept may have several important implications under *in vivo* conditions: if wandering cells, carrying cytophilic antibody, can be attracted to the origin of antigen release, whether the antigen is microbial or derived from allogenic tissue or tumour cells, their accumulation at that site would then not only depend on C activation or the formation and release of chemotactic factors by proliferating lymphocytes. This mechanism, for instance, could contribute to the development of an Arthus reaction in C4-deficient guinea pigs (refs 5 and 6); it could also be the basis for the immune-mediated emigration of neutrophils into the lumen of the small intestine, observed in pigs sensitised with BSA (ref. 7).

We had an opportunity to test our views in clinical rather than experimental conditions. A patient with a long history of dermatophytosis (*Trichophyton rubrum*) was by mistake skin-tested with an excessive dose of dermatophytin, and within 30 min developed a severe local reaction with increasing induration, erythema and pain accompanied by general malaise and fever. The reaction, which began to subside after 48 h, was Arthus-like, although there was no histological confirmation. The patient's peripheral PMNs isolated 2 and 8 weeks after the incident were strongly chemoattracted by dermatophytin, whereas PMNs from individuals negative to the skin test were completely unaffected by the antigen.

As preliminary experiments in our laboratory and elsewhere (P. C. Wilkinson, personal communication) conducted with macrophages are in agreement with the data reported here for PMNs, we believe that our concept of antigen chemotaxis may be a rather general one. We propose, therefore, as a hypothetical extension of our findings, that lymphocytes (of the B as well as the T-cell series) may also be chemoattracted to the site of the antigen that they are specifically equipped to recognise. The same signal that results in the turn-on of the cell on interaction of its surface recognition molecules with the antigen may also result in its directional migration. This hypothesis would endow the concept of immunological surveillance with an active search and guiding system instead of the chance encounter on which it is now based.

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except at the neuromuscular junction³. How nerves repress this extrajunctional AChE is not known. The possibility that they do so by signalling the muscles to contract, and that muscle contractile activity itself reduces the level of enzyme, has been tested by extracellular stimulation of muscle cultures with electrical impulses. Our results indicate that contraction reversibly reduces AChE activity of muscle cultures.

Muscle cultures were obtained from 11-day chick embryo pectoral tissue by trypsinisation and the cells were cultured in collagen-coated dishes in a medium of 10% horse serum, 2% embryo extract and 88% minimal essential medium (MEM)². The fibres exhibited spontaneous contractions and cross striations after 5 d in culture and were readily maintained for 3 weeks *in vitro*.

Electrical stimulation resulted in decreases in the AChE of the cells and the AChE released into the medium (Table 1). In each of eight experiments the AChE activity was lower for stimulated than for unstimulated cultures. The average reduction of AChE activity of the cells was 37%. The decrease observed for the AChE released by the cells was even more pronounced, averaging 61%. The total decrease in AChE of the cultures varied with a range of 30% to 90%.

Since denervation increases AChE activity in chicken muscle fibres *in situ*⁴, we investigated whether AChE activity would increase when electrical stimulation ceased. The data (Table 2) show that AChE increased after stimulation, indicating that suppression of AChE activity

Table 1 AChE levels of muscle cell cultures 48 h of stimulation

Experiment	ΔA per min per dish*	
	Cells†	Media‡
1 Unstimulated	0.62±0.05	3.21±0.40
Stimulated	0.44±0.02	1.31±0.40
2 Unstimulated	0.36±0.02	2.41±0.20
Stimulated	0.20±0.02	0.99±0.34
3 Unstimulated	0.36±0.06	1.29±0.20
Stimulated	0.22±0.04	0.68±0.40
4 Unstimulated	0.17±0.03	0.89±0.13
Stimulated	0.14±0.01	0.60±0.14
5 Unstimulated	0.19±0.02	1.27±0.20
Stimulated	0.12±0.01	0.19±0.10
6 Unstimulated	0.33±0.02	1.51±0.25
Stimulated	0.16±0.01	0.00±0.00
7 Unstimulated	0.38±0.02	1.20±0.20
Stimulated	0.12±0.01	0.00±0.00
8 Unstimulated	0.36±0.05	1.71±0.50
Stimulated	0.27±0.02	1.33±0.15
Average Unstimulated§	0.35	1.69
Stimulated	0.21	0.64

A Grass S8 stimulator was connected in series to a pair of platinum electrodes approximately 25 mm long and 3 mm wide. The electrodes were attached to the top of a culture dish and immersed in the culture media. Six dishes in series were given a 100 mm s⁻¹ train consisting of three 10-ms pulses repeated every 5 s. The voltage was approximately 30 V, the minimum that produced contractions of >75% of the fibres as determined with an inverted phase contrast microscope. Stimulation was continued for 48 h and polarity was switched frequently to minimise effects of electrolysis.

*AChE activity was determined spectrophotometrically by the Ellman assay¹⁰ using the pseudocholinesterase inhibitor iso-OMPA as in previous reports¹⁻³.

†Values are averages ± s.d. of six dishes for experiments 1 and three dishes for experiments 2-8.

‡Values are averages ± s.d. of 12 dishes for experiment 1 and six dishes for experiments 2-8.

§Statistically significant difference between unstimulated and stimulated cultures *P*<0.01 (Student's *t* test for paired variates¹¹).

Control of acetylcholinesterase by contractile activity of cultured muscle cells

THE activity of the enzyme acetylcholinesterase (AChE) is one of the many properties of skeletal muscle known to be regulated by nerves. For example, AChE is synthesised and released by chick embryo muscle fibres grown *in situ* and in culture^{1,2}. During maturation *in situ*, neural activity decreases AChE everywhere in the muscle fibres of chicks

Table 2 AChE levels of muscle cell cultures 48 h after stimulation

Experiment	Δ Cell†	Δ A per min per dish* media	Total production
1 Unstimulated	+0.21±0.14	3.09±0.30	3.30±0.44
Stimulated	+0.10±0.08	1.24±0.04	1.34±0.12
2 Unstimulated	-0.14±0.03	1.02±0.20	0.88±0.23
Stimulated	+0.15±0.05	1.23±0.30	1.38±0.35
3 Unstimulated	-0.18±0.08	0.56±0.07	0.38±0.15
Stimulated	+0.22±0.08	1.62±0.20	1.84±0.28
4 Unstimulated	0.00±0.04	0.62±0.04	0.62±0.08
Stimulated	+0.08±0.02	0.99±0.10	1.07±0.12
5 Unstimulated	0.00±0.04	1.33±0.27	1.33±0.31
Stimulated	+0.10±0.03	1.32±0.06	1.42±0.09
7 Unstimulated	-0.20±0.09	1.08±0.10	0.88±0.19
Stimulated	+0.25±0.05	2.23±0.07	2.47±0.12
8 Unstimulated	-0.10±0.09	1.09±0.20	0.99±0.29
Stimulated	+0.01±0.04	1.30±0.05	1.31±0.09
Average			
Unstimulated‡	-0.06	1.26	1.20
Stimulated	+0.13	1.42	1.55

*Values are means of $\pm$ s.d. of six dishes for experiment 1 and three dishes for experiments 2-8.

†Cell activity 48 h after stimulation minus cell activity immediately after stimulation.

‡Statistically significant difference between unstimulated and stimulated cultures $P < 0.02$ for experiments 2-8. Differences are not significant ($P > 0.10$) for experiments 1-8. (Student's t test¹¹.)

was reversible. In addition, except for the first experiment, cell AChE and release of AChE in the medium tended to be greater 48 h after stimulation for previously stimulated than for unstimulated cultures.

The increase of AChE activity after stimulation was also evidence that stimulation did not injure the cells. Furthermore, the cells were still contracting and were morphologically unchanged when observed by light microscopy after 48 h of stimulation. Also, the ability of the cultures to synthesise protein was not affected. Incorporation of ³H-leucine² (ICN Pharmaceuticals, 10 μ Ci per culture in leucine-free MEM) into trichloroacetic acid precipitates of cultures labelled for 1 h after stimulation gave an average in four experiments of 38,000 c.p.m. for stimulated cultures and 36,000 c.p.m. for unstimulated cultures.

We reasoned that if muscle activity were involved in regulating AChE, cultures unable to contract would produce more AChE than those contracting spontaneously. Tetrodotoxin (a drug that blocks excitation of the membrane of muscle fibres in culture⁵), 0.1 mg per dish, was added to 5-d-old cultures and the cells were maintained in a non-contracting state for 6 or 8 d. The cells and media were sampled every 2 d and the total AChE produced over the entire period was compared for the relaxed and the contracting cultures (Table 3). The non-contracting tetrodotoxin-treated cultures produced much larger amounts of AChE, averaging 218% the activity of the spontaneously contracting cultures.

Stimulation has also been shown to affect ACh sensitivity *in situ* and ACh receptors in culture. Drachman and Witzke⁶ and Lømo and Rosenthal⁷ reduced the spread of ACh sensitivity over the membrane of denervated rat muscles and Cohen and Fischbach¹ reduced the extrajunctional ACh receptors of embryo muscle cultures by means of electrical stimulation. When considered together, these reports and the data presented here strongly suggest that some regulatory actions of nerve on muscle are mimicked by stimulation and may be due to muscle activity, particularly with regard to extrajunctional localisation of

Table 3 Effect of tetrodotoxin on AChE of muscle cell cultures

Experiment	Δ A per min per dish accumulated activity*	Interval (d)
9 Untreated	10.5±1.1	8
TTX	29.9±2.5	
10 Untreated	14.9±1.6	8
TTX	25.4±1.0	
11 Untreated	10.4±1.1	6
TTX	20.4±1.7	
Average % TTX per untreated		218

Tetrodotoxin (TTX, 0.1 μ g per dish) was added to cultures at 5 d and maintained for the time indicated.

*Values for averages $\pm$ s.d. of five dishes for experiment 9 and four dishes for each experiments 10 and 11. Accumulated activity represents the sum of the changes in AChE activity of the cells plus the activity released into the medium during 6 or 8 d.

molecules involved in transmission at the neuromuscular junction. Whether contractions themselves are necessary or whether excitation of the muscle membrane would suffice is a matter for further investigation. There is evidence that muscle activity⁸ and cholinergic transmission⁹ may not be required for localisation of ACh receptors at the neuromuscular junction. Therefore, it is possible that there are separate regulatory mechanisms for extrajunctional and endplate AChE and ACh receptors.

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Presence in brain of a mitogenic agent promoting proliferation of myoblasts in low density culture

NORMAL animal cells in tissue culture proliferate very slowly, or not at all, when their density is less than 20 cells mm^{-2} (ref. 1). This property of density-dependent cell proliferation has long been a major obstacle to establishing clonal cultures. Conditioned media⁴ or feeder layers^{2,3} have been used to promote proliferation of single cells in culture. We have reported the presence, in bovine pituitary and brain, of a mitogenic factor, fibroblast growth factor (FGF), that can control the rate of growth of animal cells *in vitro*⁵. We report here that the proliferation of myoblasts

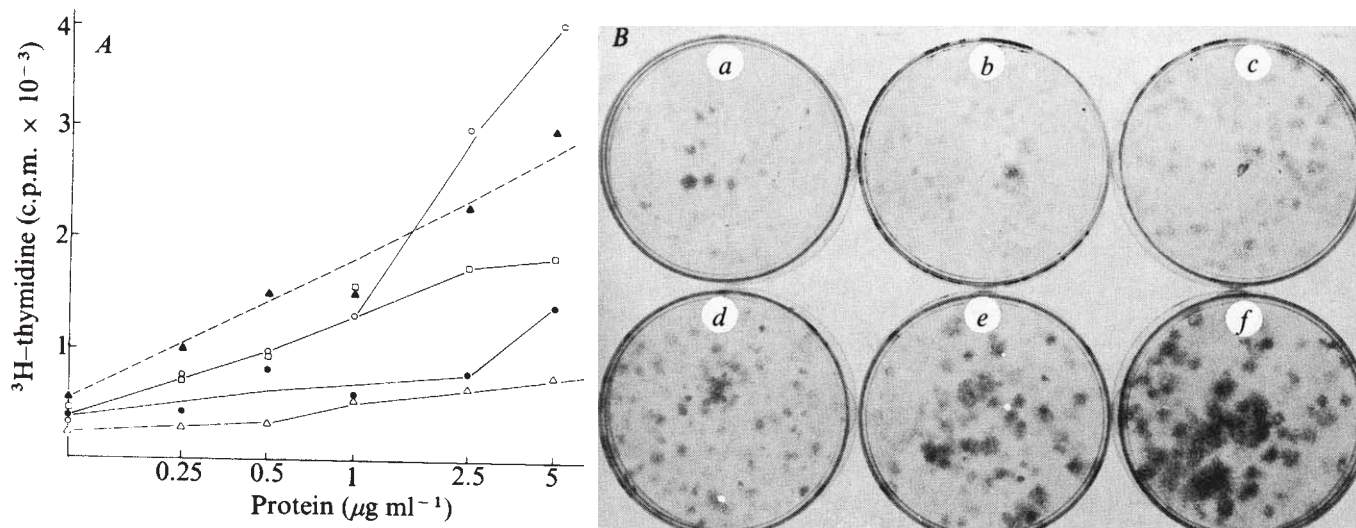


Fig. 1 *A*, DNA synthesis in BALB/c 3T3 cells in response to various concentrations of brain crude extracts prepared at diverse pH. Crude brain extracts were produced by homogenisation of bovine brains at 4 °C in 0.15 M $(\text{NH}_4)_2\text{SO}_4$. The pH was adjusted to either 3.0, 4.0, 4.5, 7.0 or 8.5 and the homogenate was stirred for 2 h at 4 °C, and centrifuged at 23,000g. The supernatant was dialysed, centrifuged and then lyophilised. FGF was prepared as described previously^{5,6}. 3T3 cells were plated in Dulbecco's modified Eagle's medium (DME) with 10% calf serum (4×10^4 cells per 35×15 mm dish with 2 ml medium per dish). After 1 d the medium was removed and replaced by DME with 0.1% calf serum. Two days later various concentrations of samples dissolved in the presence of 0.5% crystalline bovine serum albumin (to minimise nonspecific adsorption) were added to the cultures. Determinations of the incorporation of ^3H -thymidine into DNA were carried out as described previously⁹. $\circ$, pH 4.0; $\blacktriangle$, 4.5; $\square$, 3.5; $\bullet$, 7.0; $\triangle$, 8.5. *B*, Effect of brain crude extracts obtained at different pH on the proliferation of myoblasts seeded at low density. Myoblasts were obtained from foetal calf (at 3 months gestation) by trypsinisation of the thigh muscle as described previously¹². The resulting cell suspension was plated on to three 15-cm plastic tissue culture dishes (Falcon) in 1:4 DME-medium 199 supplemented with 10% foetal calf serum and 1% chick embryo extract, then incubated at 37 °C in a humidified incubator in an atmosphere of 5% CO_2 in air. After 1 d the medium was renewed and 5 $\mu\text{g ml}^{-1}$ cytochalasin B was added to select for myoblasts as described by Sanger¹³. The next day, the myoblasts were shaken loose from the dishes by gentle agitation, and the resulting suspension was centrifuged. The pellet was suspended in 1:4 DME-medium 199 with 10% horse serum. The cells were then plated at a final concentration of 300 cells per 6-cm gelatinised dish in 5 ml 1:4 DME-medium 199, with 10% horse serum. The brain crude extracts obtained at diverse pH were added 24 h later to a final concentration of 100 $\mu\text{g ml}^{-1}$. Similar numbers of clones were observed when the extract was added at the time of seeding or 1 d later. The dishes were incubated for 14 d (control) and 7 d (experimental) without a change of medium or further additions. After rinsing and fixing, the dishes were stained with crystal violet. *a*, Control; *b*, pH 3.5; *c*, 4.0; *d*, 4.5; *e*, 7.0; *f*, 8.5. Note the formation of myotubes in *e* and *f*.

seeded at low densities can be greatly accelerated by low concentrations of brain or pituitary crude extracts. This myoblast growth factor (MGF) is distinct from FGF.

The effect of brain crude extracts prepared at diverse pH on the initiation of DNA synthesis in 3T3 cells maintained in low serum is shown in Fig. 1*a*. Extracts prepared at pH 4.0 or 4.5 were the most potent, and those prepared at pH 7.0 and 8.5 were essentially free of activity. In contrast, when the diverse crude extracts were tested on sparse, primary cultures of myoblasts maintained in 10% horse serum, the most active extract was that prepared at pH 8.5. The pH 7.0 extract was less active than the pH 8.5 (Fig. 1*b*).

As the mitogenic (FGF) activity for 3T3 cells is extracted preferentially at pH 4.5, whereas the activity responsible for the growth of myoblasts maintained at very low density in the presence of 10% serum is best extracted at pH 8.5, the activities are, very likely, distinct.

Ion-exchange chromatography provided further evidence that MGF activity was distinct from FGF. When a pH 8.5 crude extract of brain was applied on a CM Sephadex column equilibrated with 0.1 M sodium phosphate, pH 6.0, MGF activity was unadsorbed. In the same conditions, the FGF activity was retained⁶. When the pH 8.5 crude extract of brain was applied on a DEAE-Sephadex column equilibrated with 0.05 M sodium phosphate, pH 7.6, MGF activity was retained and the FGF activity was unadsorbed. This indicates that MGF is a neutral polypeptide compared with FGF, which is basic.

The effect of the pH 8.5 brain crude extract on the rate of proliferation of myoblasts seeded at 300, 100 and 50 cells per 6-cm dish was measured (Fig. 2). After 14 d, little growth had taken place in the control, although the cells had been maintained in the presence of 10% horse serum. In contrast, when the myoblasts were maintained in 10%

horse serum plus 100 $\mu\text{g ml}^{-1}$ of pH 8.5 extract, extensive proliferation took place in all cases. Extensive differentiation, as reflected by the formation of myotubes, was also observed (Fig. 3). In 70% of the colonies, striated myotubes formed and in 20% of the colonies, syncytium formation was noticed. This suggests that crude extract not only promotes cell proliferation, but also promotes differentiation, as more than 75% of the nuclei were in myotubes.

When the myoblasts were maintained in the presence of FGF, proliferation was stimulated, but not to as great an extent as with crude extract of brain. Furthermore, the colonies did not differentiate into myoblasts as extensively as those with brain crude extract added. When myotubes were present in a colony they did not contain more than two to five nuclei per myotube (Fig. 3).

Pituitary crude extracts had an effect similar to that of brain crude extracts and FGF purified from pituitary had an effect similar to FGF from brain.

Chick embryo extract promoted the proliferation of myoblasts seeded at low density, although the effect was small when compared with brain crude extract (Fig. 4).

As NIH pituitary preparations, such as luteinising hormone (LH) and thyroid-stimulating hormone (TSH), have been reported to contain mitogenic contaminants^{7,8}, the effects of several NIH preparations on myoblast proliferation were compared. Of the various preparations tested, follicle-stimulating hormone (FSH) and gonadotrophic hormone had the least activity. LH of either ovine or bovine origin and TSH, however, had considerable growth-promoting activity. Insulin, which has been reported to promote cell proliferation *in vitro* when present at high concentrations, was inactive on myoblasts seeded at low density (Fig. 4).

In conclusion, we have reported that crude extract of

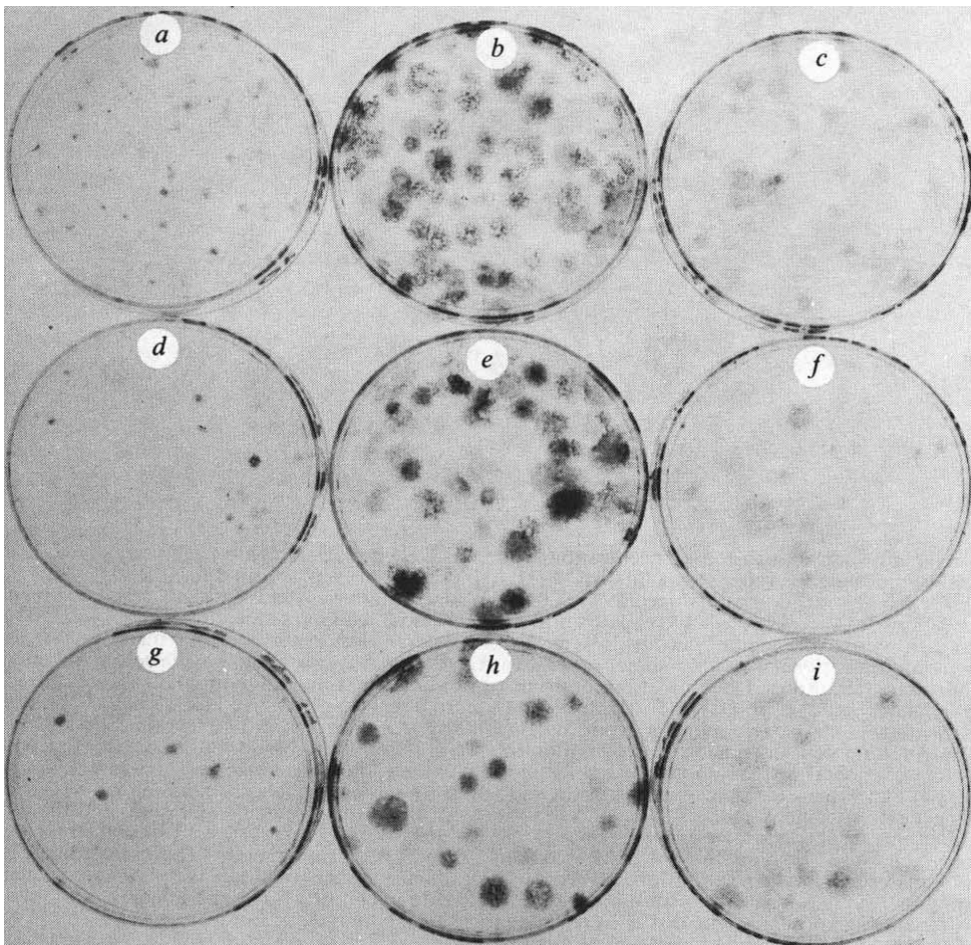


Fig. 2 Comparison of effects of brain crude extract (pH 8.5) and FGF on proliferation of myoblasts seeded at low density. Cells (300, 100 or 50) were plated in 6 cm dishes in 5 ml 1:4 DME-medium 199 with 10% horse serum. Brain crude extract ($100 \mu\text{g ml}^{-1}$) and FGF ($1 \mu\text{g ml}^{-1}$) were added 24 h later. Controls did not receive any additions and no change of medium was carried out during the incubation. Controls were incubated for 7 d. After 7 d, the myotubes in the dishes in the presence of brain crude extract started to contract rhythmically and detached, preventing further incubation. *a*, 300 cells; *b*, 300 cells plus $100 \mu\text{g ml}^{-1}$ brain extract, pH 8.5; *c*, 300 cells plus $1 \mu\text{g ml}^{-1}$ FGF; *d*, 100 cells; *e*, 100 cells plus $100 \mu\text{g ml}^{-1}$ brain extract, pH 8.5; *f*, 100 cells plus $1 \mu\text{g ml}^{-1}$ FGF; *g*, 50 cells; *h*, 50 cells plus $100 \mu\text{g ml}^{-1}$ brain extract, pH 8.5; *i*, 50 cells plus $1 \mu\text{g ml}^{-1}$ FGF. Note the formation of myotubes in *b*, *e* and *h* and their apparent absence in *c*, *f* and *i*.

brain prepared at pH 8.5 contains potent mitogenic factor(s) for myoblasts seeded at low density. The observation that those factors for myoblasts can be selectively extracted at high pH, whereas FGF, soluble at low pH, is reminiscent of the situation for polypeptide hormones; some are most soluble at low pH, whereas others are most soluble at high pH (ref.9).

In addition to its mitogenic effect, the pH 8.5 brain crude extract also promoted the formation of myotubes. In that respect it seems to act as a differentiating agent similar to those present in conditioned medium used for promoting

the fusion of chick myoblasts^{10,11}. In contrast, FGF, although it was less potent than the pH 8.5 crude extract, promoted proliferation of myoblasts but not extensive fusion. This observation agrees with the suggestion that FGF is an agent which promotes proliferation but delays fusion (our unpublished work).

The observation that, at pH 8.5, additional mitogenic agents other than FGF can be extracted from the brain may be of technical use for cloning cells or for accelerating the growth of the cells that divide slowly.

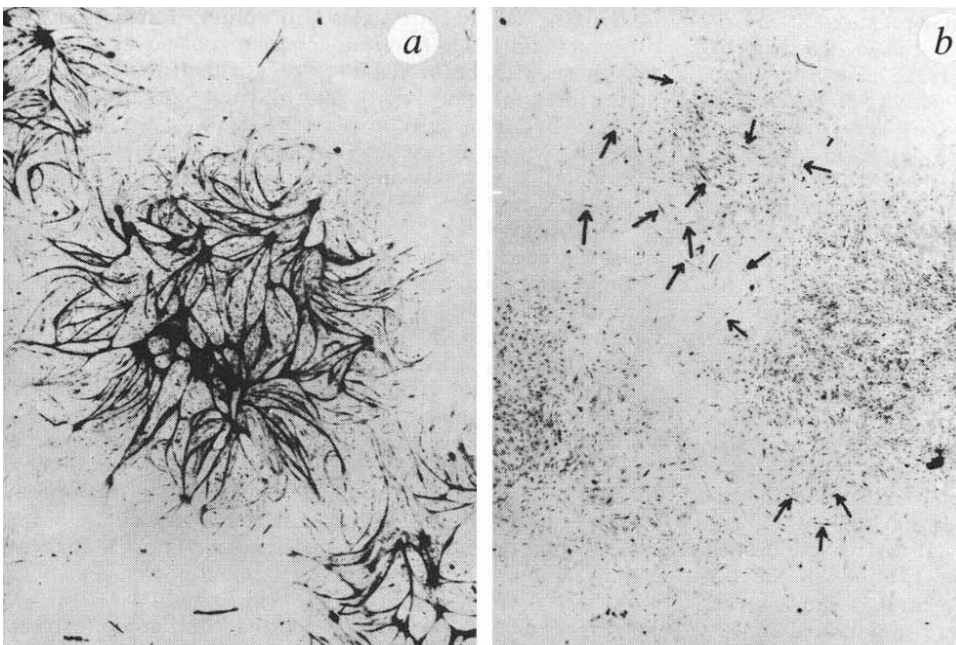


Fig. 3 Clones of myoblasts ($\times 10$) grown in presence of either brain crude extract, pH 8.5, or FGF. *a*, Clone grown in the presence of brain crude extract, pH 8.5 ($100 \mu\text{g ml}^{-1}$). Cells (300) were seeded in a 6 cm dish giving rise to colonies. Shown is a clone arising from one of these cells after 10 d. Note extensive network of myotubes, very well differentiated. Some of the myotubes contain as many as 1,000 nuclei. In some instances, presence of a loose syncytium was observed. *b*, Clone grown in the presence of FGF ($1 \mu\text{g ml}^{-1}$). Original plating was as described for *a*. Clone contained mostly single cells, with some forming short myotubes with two to three nuclei (arrows).

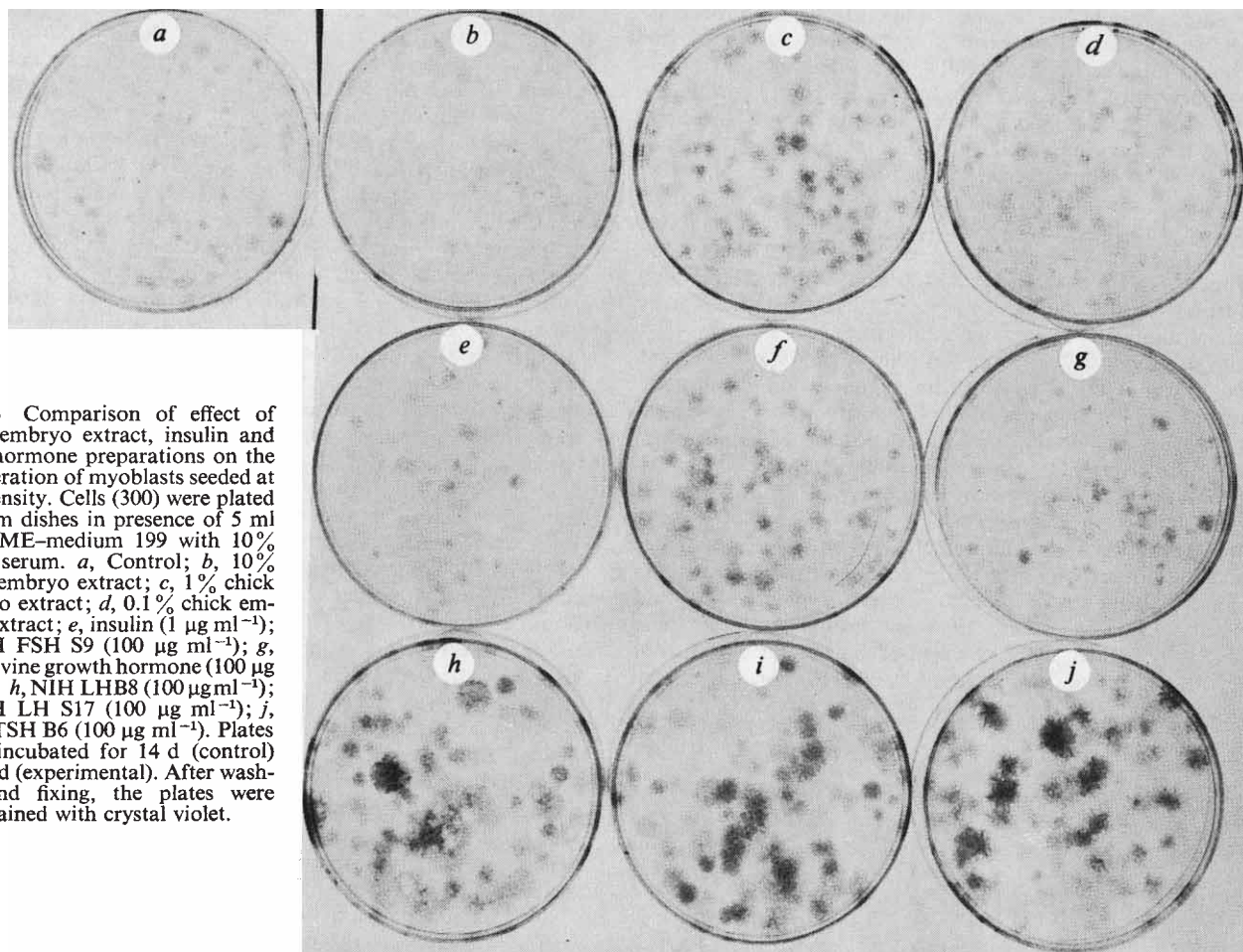


Fig. 4 Comparison of effect of chick embryo extract, insulin and NIH hormone preparations on the proliferation of myoblasts seeded at low density. Cells (300) were plated in 6 cm dishes in presence of 5 ml 1:4 DME-medium 199 with 10% horse serum. *a*, Control; *b*, 10% chick embryo extract; *c*, 1% chick embryo extract; *d*, 0.1% chick embryo extract; *e*, insulin ($1 \mu\text{g ml}^{-1}$); *f*, NIH FSH S9 ($100 \mu\text{g ml}^{-1}$); *g*, NIH ovine growth hormone ($100 \mu\text{g ml}^{-1}$); *h*, NIH LHB8 ($100 \mu\text{g ml}^{-1}$); *i*, NIH LH S17 ($100 \mu\text{g ml}^{-1}$); *j*, NIH TSH B6 ($100 \mu\text{g ml}^{-1}$). Plates were incubated for 14 d (control) and 7 d (experimental). After washing and fixing, the plates were stained with crystal violet.

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Capacity of foetal spinal cord obtained from dystrophic mice (*dy*²*J*) to promote muscle regeneration

RECENTLY Gallup and Dubowitz¹ described a series of tissue culture experiments in which they attempted to clarify the part played by the motor neurone in the pathogenesis of murine muscular dystrophy. Bishop *et al.*² had shown that muscle explants obtained from human subjects grew nor-

mally in Carell flasks. Gallup *et al.*³ reported similar results with muscle explants of trypsinised minced muscle obtained from dystrophic chick that had been grown *in vitro* on collagen-coated surfaces in Petri dishes. They were unable to differentiate between cultured normal and dystrophic muscle. But they reported significant differences between cultures of normal mouse cord with either normal or dystrophic muscle and dystrophic cord with either normal or dystrophic muscle. When contacted by ventral root neurites from normal spinal cord, the muscle cells of both normal and dystrophic muscle fused to form new fibres with well developed cross striations and peripherally located nuclei. The appearance of both normal and dystrophic muscle coupled with 'dystrophic' spinal cord was quite different. The initial cellular regenerative response was followed in most cases by degeneration and disappearance of all muscle elements, leaving only strands of connective tissue. In only three out of thirteen cultures were myotubes formed, and these failed to develop synchronised contractions.

On the basis of this evidence, Gallup and Dubowitz¹ concluded that the lesion causing murine dystrophy in muscle is primarily neurogenic.

Their results partly contradict our own observations⁴ that normal and dystrophic muscle can regenerate equally well in the presence of foetal spinal cord obtained from either normal or dystrophic mice. In an attempt to explain the apparent contradiction, we have examined the procedure applied by Gallup and Dubowitz¹. It occurred to us that Gallup and Dubowitz¹ may possibly be reporting results obtained in their small samples mainly from cultures of lumbar spinal cord. In our previous investigations we randomly coupled cross sections from all levels of the cord

with adult muscle. It seemed imperative, therefore, to determine whether the suspected neurogenic lesion of the dystrophic mutant might initially be located in the lumbosacral portion of the cord. Since the dystrophy in the *dy* mouse is progressive and starts in the muscles of the hind limbs which are innervated by lumbosacral fibres, the possibility that a difference in the procedures may be responsible for the contradictory results could not be excluded.

To test this hypothesis, cervical, thoracic and lumbar foetal cord from 15-d-old mouse embryos homozygous for dystrophy *dy^{2J}/dy^{2J}* were explanted and within 5 d coupled to normal tibialis muscle as well as to dystrophic tibialis muscle obtained from young adult mice.

Our attention was focused on the capacity of different levels of spinal cord to stimulate regeneration of muscle. In particular, the purpose of this experiment was to determine whether the lumbar and sacral portion of 'dystrophic' cord differs from cervical and thoracic cord in its ability to promote differentiation of regenerated muscle strips coupled with it in culture.

The experimental technique relied essentially on a tissue culture system in which spinal cord and muscle fragments from normal and dystrophic mice were explanted in close proximity to each other, that is, 'coupled'. Dystrophic mutant mice C57BL/6J *dy^{2J}* supplied by the Jackson Laboratory, Bar Harbor, Maine, and raised in our laboratory were used.

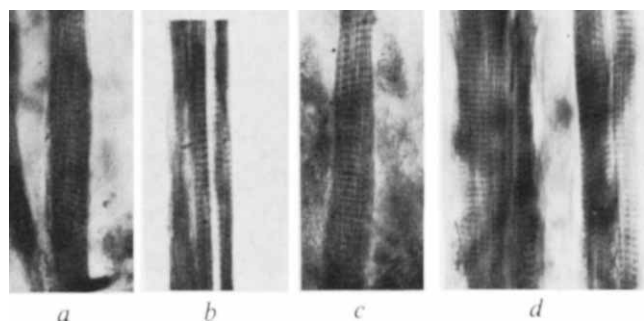


Fig. 1 *a*, Whole mount preparation of a culture of normal muscle coupled to dystrophic cord cervical level *in vitro* 50 d with phosphotungstic acid haematoxylin stained (PTAH); $\times 380$. *b*, Same as *a* coupled to dystrophic cord thoracic level, 50 d *in vitro*, stained with PTAH; $\times 380$. *c*, Same as *a* coupled to dystrophic cord lumbar level, 60 d *in vitro*, stained with PTAH; $\times 380$. *d*, Same as *a* coupled to dystrophic cord sacral level, 60 d *in vitro* stained with PTAH; $\times 380$.

Spinal cords were dissected from foetal mice aged 13–14 d *in utero*. After laminectomy, cervical and lumbar cord with its meningeal covering and attached dorsal root ganglia (DRG) were obtained from the fetuses. Complete cross sections of the spinal cord with their accompanying DRG (ref. 5) were explanted on to collagen-coated coverslips^{5,6} provided with a drop of nutrient fluid, and sealed into a Maximow double coverslip assembly. The nutrient medium which was renewed twice a week consisted of 33% human placental serum, 10% chick embryo extract (9 d), 50% Eagle's minimal essential medium (MEM), 7% balanced salt solution, and was supplemented with 600 mg% glucose and 1.32 ml⁻¹ of acromycin. Cultures were incubated at 34–35 °C in the lying-drop position, and observed regularly by bright field microscopy employing a long-working distance, $\times 40$, oil immersion objective.

Phenotypically dystrophic mice were obtained from matings between tested homozygotes for the dystrophic gene *dy^{2J}*. Young adult mice homozygous for dystrophy and normal mice served as donors for muscle explants. Strips of muscle fibres were explanted near 4- to 5-d-old spinal cord cultures^{5,6}. The following combinations were

studied: (1) normal cords and normal muscle; (2) cervical, thoracic, lumbar and sacral levels of cord obtained from foetuses homozygous for the *dy* mutations coupled to normal tibialis muscle (Fig. 1).

Normal muscle coupled with either normal or *dy* foetal spinal cord regenerated in culture. Innervation of the muscle explants led to gradual increases in fibre size and the development of cross striations. Spontaneous synchronised contraction occurred intermittently.

Fragments from all levels of the spinal cords from dystrophic foetal mice were as effective as were normal cords in inducing regeneration and innervation of muscle.

These results clarify some of the conflicting evidence reported in the literature. They confirm our earlier observations⁴, that both normal and dystrophic muscle can regenerate in the presence of either normal or dystrophic cord. These results were most recently confirmed by Parson⁷.

The conflicting observations reported by Gallup and Dubowitz¹ may be due to a statistical sampling error. Their conclusion that muscular dystrophy is determined solely by the spinal cord and that the neurones in the spinal cord are by themselves capable of producing the myopathy is far from proven.

Several investigators have reported that electron microscopy *in vitro* material revealed abnormalities in motor endplates were present, however, both in muscle fibres numbers and distortions of synaptic vesicles⁸. In cultures of normal or dystrophic muscles coupled to *dy* cord we have seen similar motor endplate abnormalities⁴. Such abnormal endplates were present, however, both in muscle fibres which displayed extensive cross striations and highly ordered myofilaments, and in muscle fibres which showed degenerative changes.

We tentatively conclude, therefore, that the primary lesion in muscular dystrophy is probably myogenic rather than neurogenic. Our experiments do not, however, permit us to decide with certainty between these two alternatives. The tissue culture set up may not be as well suited as originally anticipated to analyse these problems since there are strong indications that in the tissue culture environment both the penetrance and the expressivity of the dystrophic alleles are considerably diminished.

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Cross bridges as the major source of compliance in contracting skeletal muscle

It has been established that the elastic properties of contracting skeletal muscle are dominated by structures within the sarcomeres. We have demonstrated¹ a decrease in the instantaneous stiffness of a contracting toad sartorius at lengths greater than body length (l_0) coinciding directly with the decline in maximum isometric tetanic tension. This finding suggested that the cross bridges between actin and myosin filaments may be the major site of compliance in an active muscle, a conclusion in accordance with that reached by Huxley and Simmons² on the basis of a study of tension transients following rapid releases in single fibres of frog semitendinosus. Also in agreement with this conclusion is the work of Cleworth and Edmond³ who used a light diffraction technique to demonstrate that any change in the length of the sarcomere during isometric contraction was less than 50 Å, or less than 0.1%. These studies suggest that the cross links could be the major site of elasticity within the sarcomere, but it is possible that significant compliance resides in the unbonded thin filaments, although X-ray diffraction studies of contracting muscle⁴ indicate that the I-filament length does not increase by more than 0.02%. The experiments reported here were carried out to extend previous observations of muscle stiffness into the range of lengths below the resting length.

All experiments to be reported here were carried out using whole sartorii of the toad *Bufo bufo*. The method used for obtaining the tension-extension curve by constant velocity releases was as described previously¹, using a release velocity of $1.4\text{--}2.5\ l_0\ \text{s}^{-1}$ ($V_{\max}$ for toad muscle was measured and found to be $1.27\ l_0\ \text{s}^{-1}$).

For comparison of results from different experiments, all force values were normalised with respect to maximum tetanic force (P_0), and the changes in length were normalised with respect to body length (l_0). Plotting force against change in length produces a tension-extension curve, the linearity of a portion of which enables a linear regression

Fig. 1 Tension-extension curves from maximum tetanic tension in toad sartorius at four different lengths: ●, l_0 ; ▲, $0.8l_0$; ○, $0.72l_0$; ■, $0.68l_0$. The stiffness values for these curves are $77P_0/l_0$ (l_0 curve), $63P_0/l_0$ ($0.8l_0$ curve), $50P_0/l_0$ ($0.72l_0$ curve), $37P_0/l_0$ ($0.68l_0$ curve). For this muscle $l_0=25\ \text{mm}$; weight=67 mg.

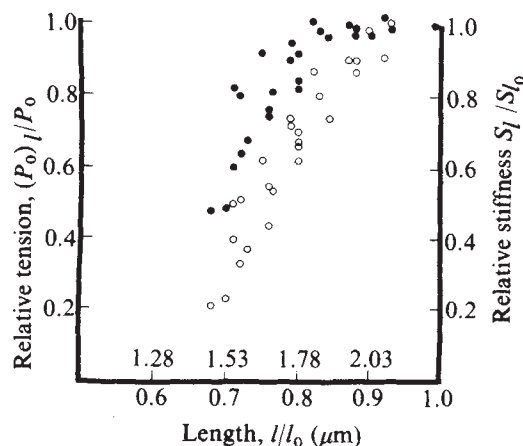
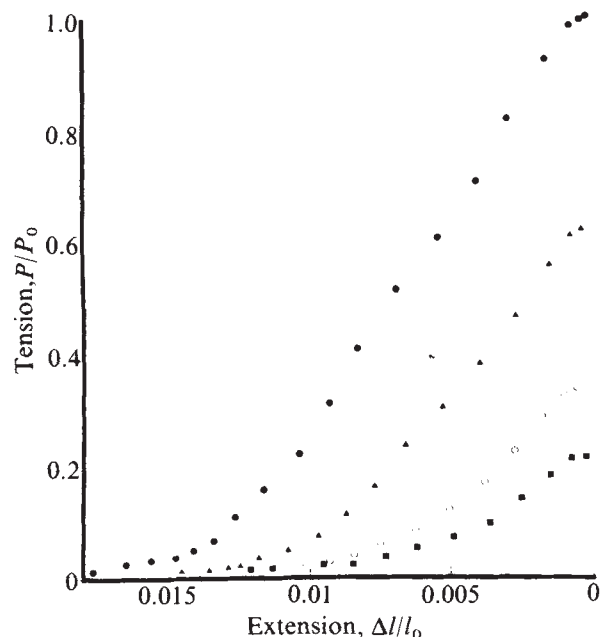


Fig. 2 Comparison of instantaneous stiffness and maximum tetanic force below body length in toad sartorii. Stiffness values (●) and tension values (○) represent the pooled data from six separate experiments. Stiffness and tension are normalised with respect to their maximum values at body length. Length is expressed as a fraction of l_0 . Abscissa: sarcomere width at the corresponding length.

analysis of the points from $0.4P_0$ to P_0 and the use of the slope of the line as a measure of muscle stiffness¹.

Four tension-extension curves obtained from the same muscle at different lengths below l_0 are shown in Fig. 1. The stiffness of the contracting muscle is decreasing as the overall muscle length decreases. Furthermore, if these curves are shifted along the horizontal axis so that the point (P_0) lies on the l_0 curve, they are not part of the same curve, indicating that the instantaneous elasticity of an active muscle as a function of load is not just determined by some passive constant element such as the tendons or the compliance of the recording equipment.

In Fig. 2, the stiffness points from six experiments are pooled and compared with the corresponding points of the tension-length relationship for the same muscles. Sarcomere widths were measured using a laser diffraction technique on whole toad sartorii.

The stiffness of the muscle remains at maximum for a short distance below the body length while the tension is falling and then, from a measured sarcomere width of about $1.82\ \mu\text{m}$, the stiffness and tension curves fall parallel to each other, with the stiffness at a given length always higher than the force. The relationship between the maximum isometric force at a given length and the stiffness at that length is shown in Fig. 3. To a first approximation, these points are linear to about $0.85P_0$ where the first sign of a decline in stiffness is observed. It was difficult to obtain values of stiffness for points below $0.2P_0$ because of the deterioration of the stimulated muscles at very short lengths⁵. The stiffness-force curve may have an intercept on the stiffness axis or may fall quite sharply to zero at points below about $0.2P_0$.

A striking feature of our experiments is the similarity of our results to the relationship between metabolic activity and force at different muscle lengths in isometric tetani. Figure 3 shows the relative change in creatine phosphate hydrolysis⁶, maintenance heat rate⁷ and stiffness with peak tetanic tension in muscles above and below l_0 . At long muscle lengths ($>l_0$) there is a very good correlation between the measured stiffness, metabolic activity and peak tetanic tension. At lengths below l_0 , the stiffness is more directly related to the creatine phosphate hydrolysis and maintenance of heat rate than to isometric tension. Moreover, the fact that the relative metabolic activity and normalised stiffness are consistently higher than the force suggests that there may be some internal force which opposes shortening below l_0 . Such a force would naturally

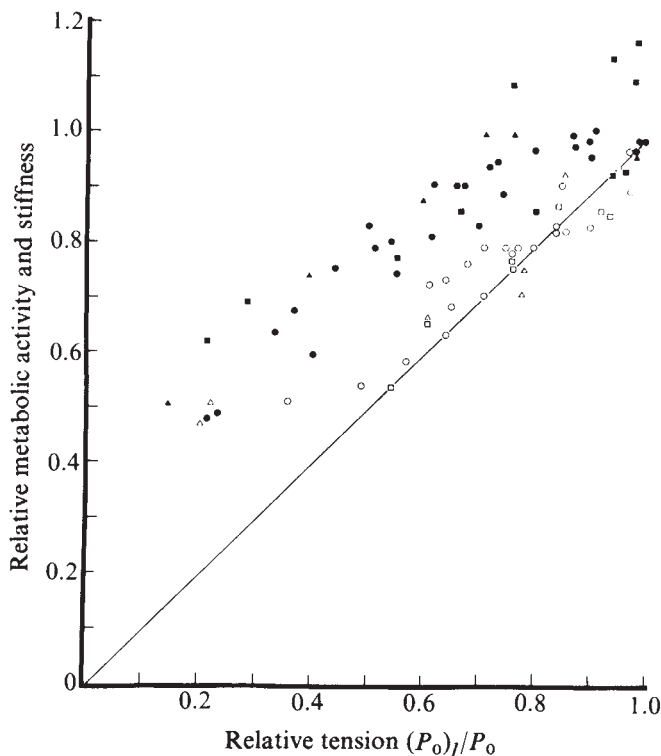


Fig. 3 Relationship between metabolic activity, instantaneous stiffness and maximum tetanic tension at different muscle lengths. Closed symbols, values for lengths below l_0 ; open symbols, lengths above l_0 . All values have been normalised with respect to their maximum value at l_0 . ■, □, Maintenance heat rate; ▲, △, creatine phosphate hydrolysis; ●, ○, instantaneous stiffness. Correlation coefficient for fit of open symbols to the line through the origin and the 1, 1 point is $r=0.91$; for closed symbols $r=0.76$.

subtract from the tension produced by the myofilaments. Evidence for such a force has been provided by Gordon *et al.*⁸ who have calculated an increase in internal osmotic pressure of 1 kg cm^{-2} in a muscle shortened from 40 to 75% of its initial length. Additional evidence has been provided by Simmons⁹ who has shown that single fibres allowed to shorten freely in solutions of increasing tonicity exhibit a definite resistance to shortening at sarcomere lengths of $2.0 \mu\text{m}$ and between 1.92 and $1.95 \mu\text{m}$. This corresponds to the range of sarcomere lengths over which the measured stiffness seems to be constant as the peak isometric tetanic tension declines.

In previous experiments which showed a decrease in the stiffness of contracting skeletal muscle at long lengths^{1,10} it seemed possible, though perhaps unlikely, that the thin filaments in the I band might be a major source of muscle compliance. If the elastic behaviour of the active muscle were dominated by the compliance of the I band, then as the sarcomere shortens and the I-band width decreases, the overall stiffness of the muscle should increase dramatically. Our work, showing that, on the contrary, the stiffness of the contracting toad sartorius decreases as the sarcomere length is reduced and as the maximum isometric tetanic tension falls, rules out such a possibility.

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Sulphydryl groups and the quinone receptor in insect olfaction and gustation

UNDERSTANDING of energy-transduction aspects of olfactory messenger-receptor interactions has been lacking¹, until the recent advances²⁻⁶ made using the chemoreceptor protein for quinone messengers, isolated from sensory nerve membranes in the antennae of *Periplaneta americana*^{7,8}. Availability of this protein for *in vitro* experiment has filled a void that existed, for several decades, between the behavioural and electrophysiological experimental capabilities in this field. With purified receptor protein available, physicochemical aspects of olfaction and gustation have become amenable to direct experiment. Here we present evidence that messenger naphthoquinones showed marked selectivity for the receptor protein when it was exposed *in vitro* to a saturating 10^{-4} M concentration of each messenger in the presence of the other Triton-solubilised proteins from the insect's antennae. The electrophoretically resolved^{7,8} receptor-containing band only constituted approximately 13.7% of the protein in the Triton-soluble fraction, but it bound more than 75% of radiolabelled messengers. The other messenger-associated radioactivity was retained at the origin of the gels or was scattered as low level DPMs among the several other resolved bands. These findings also confirm earlier experimental results⁹ on the selective binding of messenger to the receptor band as resolved in 3.5% acrylamide gels.

The possible involvement of ATPases in this chemoreception by *P. americana* has been considered previously⁹, and we now report that when the Triton-soluble proteins from the antennae were pre-incubated with saturating 10^{-4} M ouabain for 15 min before the addition of messenger 2-methyl-¹⁴C-1,4-naphthoquinone (menadione), the amount of quinone messenger bound to the receptor was not significantly reduced from that with the control at the 0.01 level of probability. Thus, active ouabain-sensitive ATPases are not involved in the primary messenger-receptor interactions in this olfactory and gustatory process.

With regard to reaction sites in the receptor, the sulphydryl reagents, N-ethyl maleimide (NEM) and iodoacetic acid (IAA), at a saturating concentration of 10^{-4} M in the Triton-soluble proteins from the antennae, showed selectivities of binding with the receptor band^{4,8} in 3.5% gels that were comparable with those of the messenger naphthoquinones. The receptor-containing band was recently shown⁴ to be exceptionally rich in sulphur-containing amino acids, and thus the now reported marked selectivity of the sulphydryl reagents seems reasonable. As a direct indication of the major role of sulphydryls as actual functional groups in the receptor for the messenger naphthoquinones, IAA binding to receptor was competitively reduced by preincubation of receptor with each of four naphthoquinones (Fig. 1). The order of this *in vitro* competitive binding at quinone concentrations of 5×10^{-6} to 10^{-5} M was the same as the order of the relative unchallenged binding of the four naphthoquinones to receptor, repellency or inhibition of feeding as measured in bioassays^{2,10}, inhibition of chemosensory neurones as

measured by electroantennograms (EAGs)⁵, binding to nerve membrane-rich density gradient fractions from antennae², shifting of the polarographic half-wave potential ($E_{1/2}$) of the isolated receptor protein³, and inhibition of ouabain-sensitive ATPases⁹. The completely compatible experimental results from all these levels of examining these messenger-receptor interactions support the final conclusion that sulphhydryls are major reaction groups (sites) for the messenger naphthoquinones' selective binding to this receptor protein.

The receptor protein was extracted from adult male *P. americana* in the same manner as detailed previously^{3,4,7,8}. The messenger naphthoquinones or sulphhydryl reagents, NEM or IAA, as used as the treatment chemical in the selective binding experiments, were added to the preparation during initial tissue maceration. In the competition experiments, the ouabain was added during initial tissue maceration, or the respective messenger naphthoquinone (Fig. 1) was introduced into uniform aliquots of the receptor extract. Each extract was then incubated for 15 min before introduction of the challenger, ¹⁴C-menadione or ¹⁴C-labelled IAA (Fig. 1). This sequence allowed each initial treatment chemical unchallenged access to the reaction sites in the receptor protein. Electrophoretic separation, the staining and quantitation of protein, the slicing of gels, and counting of radioactivity have been described⁸.

Messenger naphthoquinones showed a marked selectivity of binding for the electrophoretically resolved band of Triton-soluble protein from the antennae of *P. americana* which we previously termed the quinone receptor. Sulphhydryls were proven to be major reaction sites in the receptor. The fact that the primary receptor activity does not require an active ouabain-sensitive ATPase was proven by the failure of a preincubation of receptor with ouabain to alter significantly the binding of messenger naphthoquinone. This finding is compatible with our previous demonstration⁹ that messenger menadione does not significantly inhibit ouabain-sensitive ATPases. Thus, any disruption of chemoreception by the ouabain-sensitive ATPase inhibiting properties of naphthoquinone messengers with a significant amount of such activity (for example, juglone, 1,4-naphthoquinone, and lawsone) is strictly attributable to effects at the non-primary levels of this sensory process.

The real test of having identified critical physicochemical

parameters of this chemoreception process is the use of the obtained information as the bases for predictable alteration of the sensitivity of the system in the live animal. This has been accomplished on an extensive scale, and those results are presented elsewhere^{6,10}.

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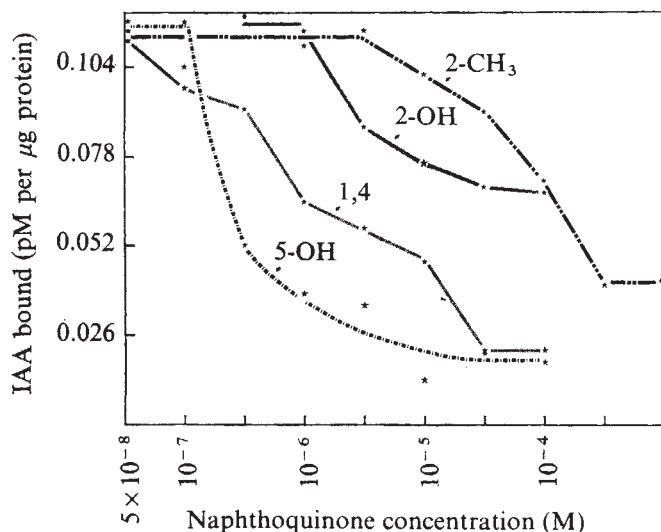
Murine sarcoma virus pseudotypes acquire a determinant specifying N or B tropism from leukaemia virus during rescue

SEVERAL classes of murine leukaemia viruses (MuLV) are recognised according to their ability to induce XC plaque formation with high or low efficiency after infection of various mouse cell lines^{1,2}. N- and B-tropic MuLVs, for example, can be distinguished on the basis of their relative ability to infect N-type or B-type mouse cells, the cells themselves differing at a single genetic locus, Fv-1 (ref. 3). Fv-1 restriction is not absolute⁴, is dominant over susceptibility in F₁ hybrids⁵, and may be mediated by a specific intracellular inhibitor^{6,7}. Studies using vesicular stomatitis virus (VSV) pseudotypes made with N- or B-tropic MuLV have shown that Fv-1 restriction occurs after virus penetration^{7,8}, although the mechanism of inhibition is not known. A clear difference exists between Fv-1 restriction of MuLV and the host range restriction of avian leukaemia virus subgroups, in which host range restriction occurs at the cell surface^{9,10}. Another class of MuLVs, NB-tropic, does not seem to be sensitive to Fv-1-mediated restriction¹.

Restriction of N- and B-tropic MuLVs is of interest as an example of host control of exogenous RNA tumour virus infection. Moreover, an understanding of Fv-1-mediated restriction may also shed light on the mechanism of control of endogenous viral genes which are known to exist, and are often unexpressed, in most, if not all, vertebrate cells¹¹.

Murine sarcoma viruses (MSVs) are a second group of murine RNA tumour viruses distinguishable from MuLV by an ability to induce sarcomas in mice and to transform a variety of cells in culture¹². Although MSV can transform cells after solitary infection, most of the MSV isolates studied to date are termed 'defective' as they are unable to replicate unless their host cell is, in addition, productively infected with MuLV (or other 'helper' leukaemia virus)^{13,14}. Certain helper-specified products seem to be required for the production of infectious MSV particles. The leukaemia virus has been shown to determine the host range^{2,15} and antigenic specificity^{16,17} of infectious MSV particles, which are, therefore, pseudotypes with respect to these properties. The ability of leukaemia viruses to specify properties of defective MSV has frequently been useful in determining

Fig. 1 Average pmol of ¹⁴C-labelled IAA subsequently bound in the electrophoresed receptor-containing protein band when 10⁻⁴ M IAA was challenged for receptor sites by a 15 min preincubation of the receptor with increasing concentrations of each of four messenger 1,4-naphthoquinones.



the function of leukaemia virus gene products. We now report that several isolates of defective MSV acquire N- and B-tropic specificities from their MuLV helper viruses.

Table 1 summarises the results of a series of focus assays using three MSV-Moloney stocks rescued from MSV-transformed BALB/3T3 non-producer cells¹³ with either N-, B-, or NB-tropic MuLV. Each MSV stock, in turn, was assayed on normal BALB/3T3 cells (B type)^{1,18}, on a continuous line of C3H cells (N type)^{4,19}, and on 3T3FL cells (dually permissive)²⁰.

As can be seen in Table 1, MSV stocks rescued with either N-tropic or B-tropic MuLV form foci with equal efficiency in 3T3FL cells when assayed in the presence of optimal NB-tropic helper virus²⁰. Also, MSV rescued with dual-tropic MuLV is equally efficient at focus formation in all three cell types.

In contrast, the stock of MSV rescued with N-tropic MuLV was almost 200 times as efficient at focus formation on N-type C3H cells as on BALB/3T3, whereas the same MSV isolate rescued with B-tropic MuLV was only 1/20 as efficient at focus formation on C3H cells (Table 1). Thus, there was nearly a 4,000-fold variation in the relative focus-forming ability of virus stocks prepared from this strain of MSV, depending on the tropism of the rescuing helper virus. (A different conclusion was reached by Yoshikura²¹, possibly because the MSV focus assay procedures used did not effectively eliminate the contribution of N- or B-tropic MuLV to focus formation by rescued MSV stocks.)

The MSV assays described above and shown in Table 1 were carried out in the presence of optimal amounts of NB-tropic MuLV 'helper' virus²². The foci scored in these assays arise as a result of the spread of progeny MSV from transformed cells; in these conditions the possibility of interactions between MuLV 'helper' virus and N- or B-tropic MSV cannot be excluded. Replicate assay plates were therefore included without added helper virus. These plates were counted 5 d after infection, and then allowed to incubate for a total of 11–13 d, at which time they were scored a second time for MSV foci. In the extended assay conditions, foci derived from MSV transformation of single cells in the absence of replicating leukaemia or sarcoma virus may be observed²³.

When the three MSV pseudotypes were tested by the longer assay procedure (Table 1), the N- and B-tropic patterns were identical to those observed above with the

same MSV stocks. MSV rescued with N-, B-, or NB-tropic MuLV exhibited the same tropism as its helper leukaemia virus. Thus, restriction of tropic MSV affects its ability to transform cells and is not altered by the presence of replicating NB-tropic MuLV in the cells used for assay. Note that restriction of N-tropic MSV by B-type cells is more pronounced than is restriction of B-tropic MSV in N-type cells, whereas the reverse pattern is observed with N- or B-tropic MuLVs¹. The restriction of B-tropic MSV in N-type cells is especially weak in assays without optimal helper virus, and, although it is a consistent finding, its significance is difficult to assess.

Foci were also counted 5 d after MSV infection in the above experiment. Each of the three MSV pseudotypes showed a typical two-hit dose-response curve when assayed in permissive cells in the absence of added helper virus (data not shown)²².

Table 1 data were obtained by titration of MSV rescued from transformed non-producer BALB/3T3 cells. A similar experiment, using dually permissive S+L- 3T3FL cells as the source of the MSV stocks, yielded nearly identical results. Therefore the Fv-1 restriction exhibited by the cell from which MSV is rescued does not seem to be a major factor in determining MSV tropism.

In addition to the Moloney isolate of MSV described above, we also tested MSV rescued from BALB/S+L cells²⁰, from K-BALB cells²⁴, and from a line of Harvey sarcoma virus-transformed 3T3FL cells recently isolated in our laboratory. All of the MSV isolates showed very nearly the same pattern of focus formation as that shown in Table 1 following rescue with N-, B- or NB-tropic MuLVs.

Our data thus show that the infectivity of MSV stocks is subject to Fv-1 restriction. MSV rescued by N- or B-tropic MuLV shows the same tropism as its helper virus, whereas dually tropic MuLV gives rise to dually tropic MSV. Cells which restrict N- or B-tropic MuLVs exhibit the same restriction to corresponding MSV pseudotypes, and the 3T3FL cell line is equally permissive to N-, B- and NB-tropic MuLV stocks as well as to their MSV derivatives (Table 1). As has been observed with N- and B-tropic MuLVs, Fv-1 restriction of MSV pseudotypes is not absolute—some MSV foci are produced in non-permissive cells.

These results indicate that MSV pseudotypes are subject to the same intracellular restriction mechanisms as are N- and B-tropic MuLVs. The tropism of the defective MSV particle is determined by its helper MuLV during the rescue

Table 1 Effect of Fv-1 restriction on the focus-forming activity of tropic MSV pseudotypes

MSV pseudotype	Assay cell line			Ratio: N/B*
	3T3FL (dually permissive)	C3H (N-type)	BALB/3T3 (B type)	
MSV(N)†	4.2 × 10 ³ ‡ (1.2 × 10 ⁴)	5.6 × 10 ³ (9.6 × 10 ³)	3.1 × 10 ¹ (2.5 × 10 ¹)	180/1 (382/1)
MSV(B)	2.2 × 10 ⁴ (1.1 × 10 ⁵)	6.4 × 10 ² (2.6 × 10 ³)	1.3 × 10 ⁴ (9.3 × 10 ³)	1/20 (1/3.6)
MSV(NB)	6.2 × 10 ⁴ (1.8 × 10 ⁵)	4.0 × 10 ⁴ (1.7 × 10 ⁵)	2.7 × 10 ⁴ (2.7 × 10 ⁴)	1.5/1 (6.3/1)
Standard§	2.8 × 10 ⁵ (1.7 × 10 ⁶)	7.4 × 10 ⁴ (ND)	5.6 × 10 ⁴ (5.5 × 10 ⁴)	1.3/1 (—)

MSV stocks were prepared following quantitative MuLV superinfection of MSV-Moloney-transformed non-producer BALB/3T3 cells. Rescue procedures were essentially as described previously²⁸, and virus pools were collected 11 d after superinfection. The N- and B-tropic MuLVs used for MSV rescue were filtered tissue culture supernatants of chronically infected permissive cell lines. Cloned seed stocks of these viruses were kindly provided by Dr J. Hartley of the NIH, Bethesda, Maryland. The IC clonal isolate of NB-tropic Moloney leukaemia virus²⁹, grown in dually permissive 3T3FL cells, was used in both the rescue experiments and as optimally diluted 'helper' virus in appropriate MSV assays. The tropism of each MuLV stock as well as the Fv-1 restriction of each cell line used was confirmed by XC plaque titration on BALB/3T3 and C3H cells³⁰. Assay plates (60 mm) were seeded with 150,000 normal BALB/3T3 (B type), 80,000 C3H (N type) or 80,000 3T3FL (dually permissive) cells. All cells were treated with DEAE-dextran (20 µg ml⁻¹) before infection.

*Ratio of MSV titre in C3H cells to MSV titre in BALB/3T3 cells.

†MSV(N) = MSV rescued with N-tropic MuLV, and so on.

‡Each number represents MSV focus-forming units ml⁻¹ based on an average of two or three assay plates within the countable range. Bracketed numbers are based on foci counted 11–13 d after infection without added helper virus; numbers not in brackets are based on foci counted 5 d after infection in the presence of optimal helper virus.

§A laboratory standard pool of MSV rescued from S+L-3T3FL cells with NB-tropic MuLV was included with each assay.

process; N or B tropism is therefore a phenotypic, helper-dependent characteristic of MSV pseudotypes. Coinfection of newly infected cells with moderate amounts (m.o.i. ~0.1) of NB-tropic helper virus during an MSV assay does not seem to alter significantly the tropic properties of MSV pseudotypes (Table 1).

The existence of N- or B-tropic MSV pseudotypes has several implications concerning the nature of Fv-1 restriction. First, the fact that MSV acquires N or B tropism from its MuLV helper implies that the determinant present in leukaemia virus particles which specifies N or B tropism is transferable to MSV during the rescue process. Once transferred, this determinant does not become an integral part of the MSV genome. The determinant of N or B tropism may be responsible for either resistance or sensitivity to Fv-1 restriction, and NB-tropic MuLV may contribute either two determinants or none at all to its defective MSV pseudotype during rescue. The factor responsible for the SV1-mediated MSV tropism is very likely distinct from the helper-derived envelope glycoprotein(s) responsible for the antigenic specificity and interspecies host range of MSV pseudotypes, as the Fv-1 restriction mechanism acts after virus penetration of resistant cells, whereas envelope glycoproteins are involved primarily in virus adsorption²⁵. The determinant of MSV tropism may be analogous to the helper-dependent factor required for MSV transformation²⁶, found using MSV rescued by certain temperature-sensitive mutants of MuLV.

Second, MSV pseudotypes are restricted in non-permissive cells not only in their ability to replicate, but also in their ability to transform cells (Table 1). Therefore, while restriction of N- and B-tropic viruses in non-permissive cells involves the production of infectious progeny, it also extends to the expression of an MSV gene product which functions in cell transformation, and not, presumably, in leukaemia virus replication. Restriction associated with the Fv-1 locus may thus involve the simultaneous repression of several viral gene products normally produced after integration of viral information into host cell genetic material²⁷, or restriction may be the result of an inhibition of the integration process itself. Molecular studies with tropic MSV in permissive and non-permissive cells could distinguish between these two possibilities.

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Note added in proof: The resistance of BALB/3T3 cells to transformation by MSV (N) can be abrogated by the addition of large amounts of N-tropic MuLV, but not by comparable amounts of B-tropic or NB-tropic MuLV.

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Induction of erythroid leukaemia by Harvey and Kirsten sarcoma viruses

SEVERAL RNA tumour viruses including the Friend¹, Rauscher² and Kirsten (MLV-Ki)³ strains cause erythroid leukaemia in mice. These agents do not transform cells *in vitro*, but provide a helper activity for the replication of defective transforming sarcoma viruses. The Kirsten sarcoma virus (KiSV)⁴ was isolated from a rat inoculated with MLV-Ki, whereas the Harvey sarcoma virus (HaSV)⁵ was recovered from a rat infected with the non-transforming Moloney leukaemia helper virus (MLV-M)⁶. Both sarcoma viruses contain common rat genetic sequences^{7,8}.

Stocks of both KiSV and HaSV cause erythroid leukaemia and solid tumours in mice^{4,9,10}. The agent responsible for inducing the erythroid leukaemia has not, however, been identified. Recently, clonal KiSV- and HaSV-transformed non-producers, free of associated leukaemia virus, have been isolated *in vitro*¹¹⁻¹³. We have rescued these defective sarcoma viruses with MLV-M and now show that these transforming viruses cause erythroid leukaemia in mice.

Cells were maintained in Dulbecco's modified Eagle's medium containing 4.5 g l⁻¹ glucose and 10% calf serum (Colorado Serum Company). The establishment of the non-transformed cell lines, NIH/3T3 (ref. 14), NRK (ref. 15), and BALB/c-3T3 (ref. 16), and the isolation of HaSV- and KiSV-transformed non-producer cells¹¹⁻¹³ have been described. A clonal isolate of MLV-M (ref. 17) was grown on NIH/3T3 cells. MLV-M was quantitated by its ability to induce syncytia formation in XC cells¹⁸. About 10⁶ non-producer cells were transferred to a Falcon flask (area, 75 cm²) containing 4 µg ml⁻¹ polybrene (Aldrich) and infected 24 h later with 10⁷ XC units MLV-M. Infected or mock-infected cells were passaged for 2 weeks. To prepare virus stock, 10⁶ cells were transferred to a 75 cm² flask; tissue culture fluids were collected 24 h later and stored at -70 °C. The *in vitro* assay for the replication of the transforming activity on NIH/3T3 cells has been described¹⁹. To induce erythroid leukaemia, virus stock was passed through a membrane filter (pore size, 0.45 µm) and 0.1 ml was inoculated intraperitoneally into newborn Swiss mice. Three to four weeks later, the animals were killed and autopsied. Spleens were sectioned sagittally and touch preparations were made and stained with the Wright-Giemsa stain or benzidine. The spleens were then fixed²⁰ and the uncut surface examined for erythroblastic foci²¹. Solid tumours were identified grossly and further characterised after staining with haematoxylin and eosin²⁰.

MLV-M-infected HaSV- or KiSV-transformed non-producers yielded a virus that transformed NIH/3T3 and caused erythroid leukaemia (Table 1). MLV-M did not transform NIH/3T3 or cause erythroid leukaemia, nor did medium conditioned by the non-producers. Thus, the HaSV and KiSV caused erythroid leukaemia because their transforming

Table 1 Rescue of the transforming erythroid leukaemia-sarcoma viruses

Cell line	Addition of MLV-M	Replication of transforming activity	Erythroid leukaemia	Recovery of transforming virus from spleens
KiSV-NIH	No	—	—	ND
	Yes	+	+	+
KiSV-BALB	No	—	—	ND
	Yes	+	+	+
KiSV-NRK	No	—	—	ND
	Yes	+	+	+
HaSV-NIH	No	—	—	ND
	Yes	+	+	+
NIH/3T3	No	—	—	ND
	Yes	—	—*	—

One day after addition of MLV-M, the infected or mock-infected KiSV-NRK were cocultivated with 10^6 NIH/3T3 cells. Virus stocks were prepared from the medium overlaying these and other cultures as described. To assay for the recovery of transforming virus, spleens were shredded through a wire grid and a 10% cell suspension made in medium containing calf serum. The cells were removed by low speed centrifugation; the supernatant was passed through a 0.45 μ m membrane filter and assayed for transforming activity on NIH/3T3 cells¹⁹.

*Some of the mice inoculated with MLV-M developed an erythroid response, which could be noted in the spleen at 1 month of age. This response could be readily differentiated from erythroid leukaemia: the cells matured normally and the predominant nucleated erythroid cell was the orthochromic normoblast; there was no evidence of haemorrhage; and discrete foci were not evident. A similar erythroid response has been noted with the Friend helper virus²⁵. Approximately 80% of these mice eventually developed the thymic tumours of Moloney leukaemia 3 months after inoculation.

ND, not determined.

activity could not be separated from their leukaemogenic activity.

A virus recovered from the spleens of the erythroid leukaemic mice transformed NIH/3T3 cells (Table 1). These transformed cells had the typical compact and piled-up appearance of KiSV- or HaSV-transformed NIH/3T3. MLV-M was also present in these spleens and was detected by the XC cell test¹⁸.

Animals with erythroblastic leukaemia had grossly enlarged spleens with many pale, ill-defined erythroblastic foci²¹. These spleens often ruptured causing the death of the animal. More than 30% of the cells in areas of

erythroid leukaemia were proerythroblasts or basophilic erythroblasts, and occasional trinucleate forms were seen. There was also an increased number of more mature erythroid cells that gave a positive benzidine stain for haemoglobin. Foci of erythroid leukaemia could also often be identified in the liver.

Some of the animals inoculated with HaSV (MLV-M) or KiSV (MLV-M) also developed solid tumours. These tumours (usually rhabdomyosarcomas or fibrosarcomas) grew slowly and did not kill the host. To compare the frequency of erythroid leukaemia with that of solid tumours, animals were inoculated with serial dilutions of virus stock. At all dilutions that caused disease, the incidence of erythroid leukaemia was greater than that of solid tumours (Fig. 1). Approximately 10^2 transforming units of HaSV or KiSV caused microscopic foci of erythroid leukaemia in 50% of the inoculated animals, while 10 transforming units did not cause erythroid leukaemia. HaSV (MLV-M) and KiSV (MLV-M) caused erythroid leukaemia in Swiss, BALB/c and C57BL/6 newborn mice.

Four defective type C viruses with transforming activity for 3T3 (Abelson virus^{19,21}, Moloney sarcoma virus^{11,22}, HaSV and KiSV) have been isolated from rodents. One of these agents, the Abelson virus, causes a lymphoid leukaemia of B or null cell origin^{23,24}, whereas HaSV and KiSV each cause erythroid leukaemia. The transforming activity of these three defective viruses is probably responsible for affecting the growth control of haematopoietic cells to induce leukaemia. Defective transforming viruses recovered from other species may also cause leukaemia.

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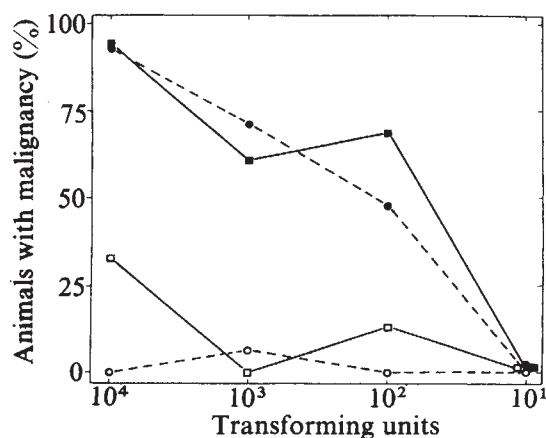
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Fig. 1 Assay of virus stock for the induction of erythroid leukaemia or sarcomas in newborn Swiss mice. Virus stock was prepared by rescuing the transforming agents from HaSV-transformed NIH/3T3 or KiSV-transformed BALB/c-3T3 with MLV-M. Both stocks had a titre of about 10^5 transforming units and 10^6 XC units ml^{-1} . Approximately 20 mice were inoculated with each dilution of 0.45 μ m filtered virus stock. □, ■, HaSV (MLV-M); ○, ●, KiSV(MLV-M). □, ○, solid tumour; ■, ●, erythroid leukaemia.



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Transcriptional events during early phase of germination of rice embryos

GERMINATION transfers a metabolically inert embryo into an active state of growth and development. The presence of conserved mRNAs has been demonstrated in different species of eggs and seeds¹⁻⁴. In rice embryos, germination was shown to be independent of the synthesis of RNA up to 18–24 h after the start of imbibition⁵, although RNA synthesis was detected as early as 9 h after the start of imbibition. In this report, the sequence of the transcriptional events taking place during the early phase of the germination of rice embryos are presented.

Rice seeds (*Oryza sativa* L. IR-8) were germinated for different periods⁵, the embryos were excised, incubated with the radioactive precursor, homogenised, and the ribosomes were separated by sucrose density gradient centrifugation⁶. The amounts of ribosomes, ³²P-RNA and ¹⁴C-protein associated with monosomes at different stages of germination are shown in Table 1. The increase in the radioactivities of ¹⁴C-amino acids and ³²P-orthophosphate associated with monosomes of embryos germinated for 12–24 h was reflected in the twofold increase in the amount of monosomes at 24 h. This indicated the synthesis of ribosomal components and maturation of ribosomes by 24 h of germination. The incorporation of ³²P-orthophosphate at 6 h was negligible, whereas it increased by sevenfold by 12 h of germination.

The ³²P-RNA isolated from the microsomes of embryos excised from seeds germinated for 12 and 24 h in presence of ³²P-orthophosphate were analysed by 2.6% polyacrylamide gel electrophoresis⁷ to investigate the synthesis of rRNA (Fig. 1). Both the 12 and 24 h samples showed the two rRNA peaks (I and II) indicating synthesis. These results imply that the rRNAs were synthesised in the early phase (0–12 h) and were retained during the entire experimental period (24 h). No significant incorporation of ³²P-orthophosphate into microsomes could be detected,

Fig. 1 Analysis of ³²P-RNA from microsomes by SDS-polyacrylamide gel electrophoresis. ³²P-RNA isolated from microsomal fraction of embryos excised from seeds grown in ³²P-orthophosphate for 12 (●) or 24 h (■) was dissolved in electrophoresis buffer (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, and 0.2% SDS adjusted to pH 7.8) and 0.1 ml (46,000 c.p.m.) was applied on to 2.6% gel and subjected to electrophoresis at 5 mA per tube for 75 min. The gel was dried between filter paper folds, cut into 1 mm thick strips with the paper and counted. ¹⁴C-labelled RNA from *Escherichia coli* was used as standard.

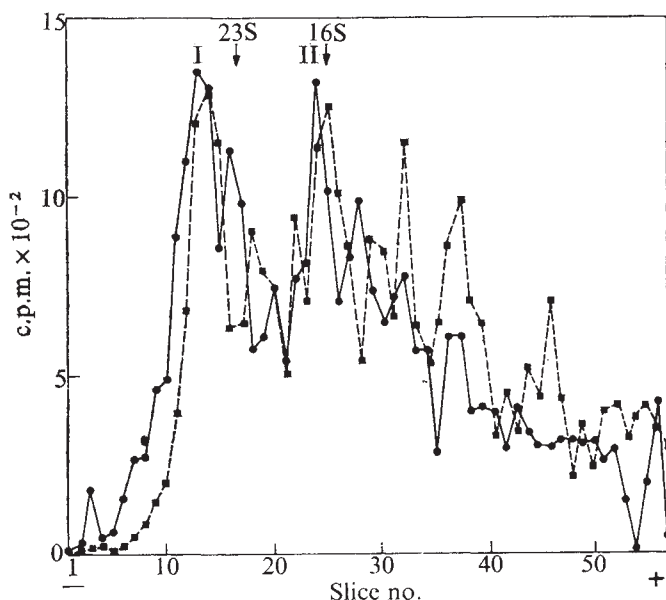


Table 1 Quantity of ribosomes, ³²P-RNA and ¹⁴C-protein at different stages of germination

Germination (h)	Ribosome (A_{260})	³² P-RNA c.p.m.	¹⁴ C-Protein
6	0.30	1,800	9,200
12	0.36	12,500	10,900
24	0.63	44,200	—

Embryos (100) at different stages of germination were homogenised in 3.5 ml 25 mM Tris HCl, pH 7.4, 10 mM MgCl₂, 5 mM β -mercaptoethanol and 0.25 M sucrose with sand in a mortar at 4 °C, centrifuged at 2,500g for 5 min; the supernatant fraction was further centrifuged at 12,000g for 15 min. Supernatant fraction (1 ml) was layered on 27.2 ml 15–30% sucrose gradient and spun at 25,000 r.p.m. in a SW-25.1 rotor for 3 h at 4 °C. Tubes were punctured and fractions of 50 drops were collected. Quantity of ribosomes was estimated by absorbance at 260 nm. For incorporation studies, the embryos were incubated in 2.5 ml 0.1 M Tris-HCl pH 7.8, 1% glucose and 50 μ g ml⁻¹ chloramphenicol in presence of ³²P-orthophosphate (60 μ Ci) or ¹⁴C-algal protein hydrolysate (10 μ Ci) at 30 °C for 2 h and the ribosomes were separated as before. Each fraction was made 10% with respect to TCA and the precipitate was washed free of lipids on glass fibre filters and counted⁸. Radioactivity under monosome peak represented ³²P-labelled RNAs or ¹⁴C-labelled proteins associated with ribosomes.

however, when the seeds were germinated for 6 h in presence of ³²P-orthophosphate. This observation and the data presented in the Table 1 indicate that rRNA appears in the cytoplasm between 6 and 12 h of germination. In wheat embryos the mature ribosomes have been detected only after 6 h of germination⁹.

Although the 24 h labelled ³²P-RNA isolated from microsomes also includes the RNA synthesised up to 12 h, there are differences in the two patterns (Fig. 1). Some new peaks of radioactivity (<16S) did appear by 24 h of germination, indicating the synthesis of a new species of RNA.

The base compositions of ³²P-RNAs isolated from embryos labelled for 1 h at 12, 18 and 24 h of germination are given in Table 2. The G+C/A+U ratio of 1.39 of the pulse-labelled RNA at 12 h of germination indicated the predominant synthesis of rRNA during 0–12 h. RNAs synthesised during the later periods have G+C/A+U ratios less than one, indicating their DNA-like nature. The synthesis of RNAs rich in G+U has been reported during the germination of wheat embryos⁹. The base composition of the pulse-labelled RNAs in rice was identical with the conserved mRNA in embryos⁴ of wheat seeds and RNAs synthesised during germination⁹. rRNAs are synthesised in rice embryos before the synthesis of mRNA and may have a role in the transport of the mRNAs from the nucleus to cytoplasm^{10,11}. The synthesis of mRNA itself takes place after the first cycle of DNA synthesis, the latter being maximum at 12–18 h (unpublished). Analysis of the post-microsomal supernatant ³²P-RNA on 10% sodium dodecyl sulphate (SDS)-polyacrylamide gel showed the incorpora-

Table 2 Base composition of pulse-labelled RNAs at different stages of germination

Germination (h)	% Total				G+C/A+U
	GMP	UMP	AMP	CMP	
12	35.0	21.5	21.1	21.2	1.39
18	25.3	26.7	25.3	22.5	0.94
24	29.9	33.8	18.9	17.9	0.91

Embryos (500) at different stages of germination were labelled for 1 h with ³²P-orthophosphate (160 μ Ci) in 3.5 ml incubation medium (Table 1), homogenised in 6 ml 10 mM Tris-HCl, pH 7.8, 20 mM KCl and 10 mM MgCl₂ and 0.1 M Na₂HPO₄. Homogenate was centrifuged at 1,500g for 10 min and ³²P-RNA was isolated from the supernatant fraction⁸. RNA was hydrolysed and the nucleotides were separated by electrophoresis on Whatman no. 1 paper at 2,000V for 105 min in pyridine acetate buffer, pH 3.5, according to the method of Sebrings¹². Nucleotides were identified by using internal standards and quantitated by the amounts of radioactivity.

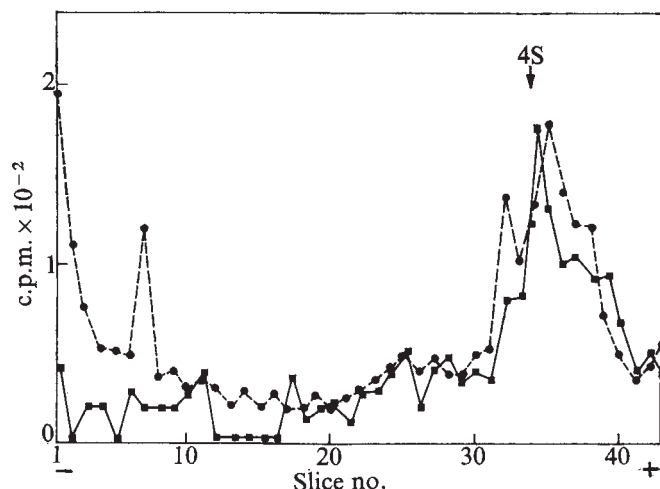
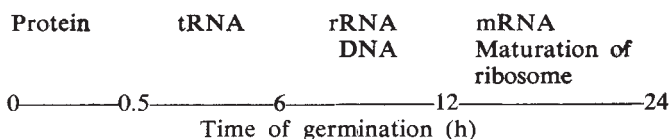


Fig. 2 Analysis of ^{32}P -RNA from postmicrosomal supernatant fraction by SDS-polyacrylamide gel electrophoresis. ^{32}P -RNA from postmicrosomal fraction (■, 6 h; ●, 12 h) was isolated, dissolved in electrophoresis buffer (Fig. 1) and 0.1 ml (2,400 c.p.m.) was applied on 10% gel and subjected to electrophoresis at 5 mA per tube for 3.5 h. Gels were cut into 2 mm thick slices, dried on filter paper disks and counted. ^{35}S -labelled tRNA from *Pseudomonas aeruginosa* was used as standard.

tion of ^{32}P -orthophosphate into 4S RNA as early as 6 h of germination (Fig. 2). This may either represent the synthesis and/or the turnover of CCA ends of tRNAs.

From the data presented above and from the published results⁸, commencement of the synthesis of various macromolecules during the early phase of the germination of rice embryos are represented as:



This scheme is at variance with that reported by Dobrzanska *et al.*⁹, in which the mRNA synthesis takes place from the beginning of the germination of wheat embryos after presoaking the seeds for 8 h at 2 °C. When wheat seeds were germinated without presoaking, however, synthesis of rRNAs was found to be the earliest transcriptional event⁸.

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Polyribosome formation in early wheat embryo germination independent of either transcription or polyadenylation

EARLY germination of seed embryos is characterised by a rapid increase in polyribosome content and rate of protein synthesis^{1,2}, and there is evidence³⁻⁸ that these events are a consequence of the mobilisation of preformed messenger RNA⁹. We observed that new mRNA synthesis could be measured at this time of rapid polyribosome formation¹⁰. Using cordycepin and α -amanitin, inhibitors of this mRNA synthesis, we have now confirmed that preformed mRNA has an important role in early germination, and have found

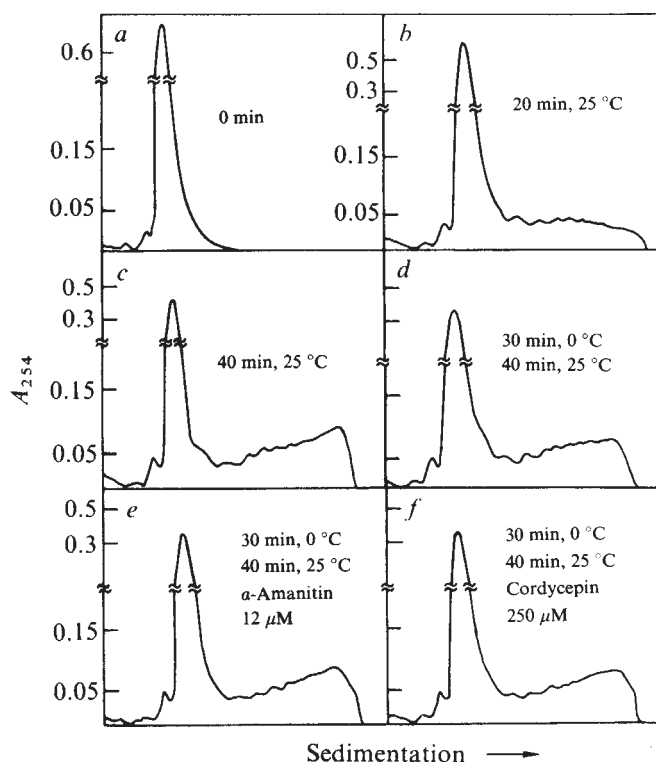


Fig. 1 Polyribosome formation during early germination of wheat embryos and the effect of RNA inhibitors. Embryos (125 mg) imbibed in 1.6 ml water either directly at 25 °C (a, 0 min; b, 20 min; c, 40 min) or at 1 °C for 30 min followed by 40 min at 25 °C (d, control; e, 12 μM α -amanitin; f, 250 μM cordycepin) were frozen in dry ice. After grinding with a mortar and pestle cooled in dry ice, the "dry ice powder" of the cells was homogenised in a Dull glass homogeniser with 7 ml of isolation media (0.25 M sucrose, 20 mM KCl, 2 mM MgCl_2 , 40 mM Tris-HCl pH 7.6, 5 mM mercaptoethanol), cleared by centrifugation (23,000g for 10 min), layered over 2 ml of a cushion of 1.8 M sucrose, 20 mM KCl, 2 mM MgCl_2 , 40 mM Tris-HCl pH 7.6, 5 mM mercaptoethanol, and centrifuged for 60 min at 150,000g in a Spinco Ti 50 rotor. (The isolation through the cushion removes some of the monosomes from the ribosome pellet¹¹. Nevertheless, it is particularly useful both in excluding ribonucleoprotein particles of non-polysomal origin and in increasing the accuracy of the analysis of the change in polyribosome content.) The resulting pellet was suspended in 0.8 ml of 20 mM KCl, 1 mM MgCl_2 , 40 mM Tris-HCl pH 7.6, 1 mM dithiothreitol and clarified by centrifugation for 10 min at 23,000g. A volume containing 150 μg RNA was layered on a 15–38% linear sucrose gradient over a 1.5 M sucrose cushion (both gradient and cushion solutions contained 20 mM KCl, 5 mM MgCl_2 , 50 mM Tris-HCl pH 7.6, 1 mM dithiothreitol) and centrifuged for 45 min at 230,000g in a SW50.1 rotor. The gradients were fractionated using a modified ISCO density gradient fractionator with continual monitoring of absorbance at 254 nm. The prominent peak of absorbance is that of 80S ribosomes. The polyribosome content of the six preparations shown are 3, 22, 55, 46, 42, and 44%, respectively.

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Table 1 Effect of cordycepin and α -amanitin on uridine incorporation into RNA of ribosomes during rapid polyribosome formation

	Uptake $\times 10^6$ (c.p.m. per 250 mg embryo)	Acid-insoluble radioactivity (c.p.m./mg RNA)	Inhibition (%)
	2.6	6,592	
+ cordycepin (250 μ M)	3.2	1,736	73
+ α -amanitin (12 μ M)	2.7	471	93

Embryos (125 mg) preimbibed either water, cordycepin (250 μ M) or α -amanitin (12 μ M) for 30 min at 0 °C and were then transferred to a similar solution containing either water or the inhibitors, and 3 H-uridine (150 μ Ci) for 40 min at 25 °C. Two batches of embryos were combined; ribosomes were isolated as in Fig. 1; RNA was extracted as in Table 3, and the acid-insoluble radioactivity was determined.

that polyribosome formation at this time is independent of both transcription and of polyadenylation.

Figure 1a-c shows a time course of polyribosome formation in wheat embryos exposed to water at 25 °C. At 20 min considerable mRNA was found in polyribosomes, and by 40 min polyribosomes of maximal size predominated. With some preparations there was no further increase in poly-

tial inhibition of the ultimate increase in protein synthetic capacity. Nevertheless, the time course of polyribosome formation (Fig. 1d) was identical to that at 25 °C. In the presence of either cordycepin (250 μ M) or α -amanitin (12 μ M), polyribosome formation was unaffected (Fig. 1e and f). Table 1 shows that RNA synthesis during the period of rapid polyribosome formation was inhibited about 70% by cordycepin and more than 90% by α -amanitin. Thus early polyribosome formation is essentially independent of RNA synthesis.

The effectiveness of the inhibitors made possible a test of the involvement of polyadenylation in the recruitment of preformed mRNA, an idea first suggested for the fertilised sea urchin egg^{12,13}, and recently advanced in studies of enzyme formation in germinating cotton seed cotyledons¹⁴. We compared the relative incorporation of 3 H-uridine and 14 C-adenosine in the presence and absence of the inhibitors. Both these precursors can be expected to label the internal transcribed component of the mRNA, while only adenosine labels the poly(A) tract at the 3' terminus. If polyadenylation of preformed mRNA were a prerequisite for polyribosome formation, it would not be inhibited by cordycepin or α -amanitin (as neither affects polyribosome formation, Fig. 1), and the process would be revealed by a substantial retention of adenosine incorporation, particularly in relation to that of uridine.*

In practice, the labelled RNA was fractionated into

Table 2 Relative inhibition by cordycepin and α -amanitin of uridine and adenosine incorporation into mRNA

	3 H-Uridine		14 C-Adenosine	
	Acid-insoluble Radioactivity (c.p.m./mg RNA)	Inhibition	Acid-insoluble Radioactivity (c.p.m./mg RNA)	Inhibition
poly(A)---				
	1,263	—	934	—
+ cordycepin (250 μ M)	635	50%	657	30%
+ α amanitin (12 μ M)	280	78%	245	74%
poly(A)+				
	3,435	—	2,615	—
+ cordycepin (250 μ M)	592	83%	480	82%
+ α amanitin (12 μ M)	131	96%	194	93%

Embryos imbibed and were incubated as in Table 1 except that the incubation medium contained 3 H-uridine (150 μ Ci) and 14 C-adenosine (15 μ Ci). The RNA extracted from the ribosomes was fractionated on oligo(dT)-cellulose columns¹⁵ into non-adsorbing (poly(A)-) and adsorbing (poly(A)+) components. Each fraction was precipitated with 5% trichloroacetic acid, collected on glass fibre filters and counted.

ribosome content for up to 5 h of germination whereas with other preparations we found increases of up to 15%. In preliminary trials with inhibitors, we found that RNA synthesis could be inhibited maximally if the embryos had imbibed the inhibitors at 0 °C. In these conditions (30 min at 0 °C) no polyribosomes were formed. Furthermore, no RNA was synthesised during this period. Preimbibition in the cold caused some diminution of polyribosome formation during subsequent exposure to 25 °C (compare percentage polyribosomes of Fig. 1c and d), as well as a par-

poly(A)- and poly(A)+ RNA, the latter providing an enrichment of radioactivity incorporated into mRNA. The data obtained (Table 2) show that the presence of the inhibitors, both of which effectively suppress incorporation into mRNA, does not cause a difference in the relative incorporation of the two precursors. We therefore conclude either that there is no significant polyadenylation of the preformed mRNA, or that the inhibitors are as effective in suppressing poly(A) synthesis as they are in preventing transcription. The latter alternative would allow polyadeny-

*This expectation is valid only if the amount of preformed mRNA entering the polyribosomes is at least of the same order of magnitude as that synthesised in the period of analysis. The RNA content of polysomes after 40 min, as determined from changes in absorbance (Fig. 1), is 100 μ g per 125 mg of embryo. Based on an average molecular weight of 6×10^5 for mRNA and an mRNA concentration of 2% in polyribosomal RNA, this represents a net increase of 3.3 pmol of mRNA. The adenosine incorporation into mRNA (Table 2) was 750 c.p.m. per 125 mg embryo. The uptake of 1×10^6 c.p.m. 14 C-adenosine into the trichloroacetic acid soluble pool (data from the experiment of Table 2), and the ATP content of 150 nmol (ref. 15 and unpublished observations of Cheung, C. P.), both per 125 mg of embryo, together with unpublished observations (Obendorf,

R. A.) showing that within 30 min of exposure, 45% of radioactive adenosine taken up by embryos is in ATP, enable the determination of the specific activity of the pool ATP as 3 c.p.m. pmol⁻¹. Again, based on a molecular weight of 6×10^5 for mRNA and a tract of 100 adenosine residues (molecular weight 30,000) at the 3' terminus, we have a total of 575 pmol adenosine per pmol of mRNA. The incorporation data calculate to 250 nmol of adenosine incorporated or a net synthesis of 0.4 pmol of mRNA. This value *per se* suggests that new synthesis could not account for the mRNA mobilised into polyribosomes. It clearly establishes that polyadenylation of preformed mRNA (in the presence of the inhibitors) would be easily detected, if it occurred.

Table 3 Poly(A) content of dry and imbibed wheat embryos

$\mu\text{g RNA}$	$^3\text{H-poly(U)}$ hybridised (ng)	
	Dry	Imbibed
6	4	4
11	13	9
28	32	24
55	45	46

Samples of dry and 40-min imbibed embryos (125 mg) were converted into dry ice powders. The powder was suspended in 3.0 ml of extraction solution (0.1 M sodium acetate, 0.1 M NaCl, 50 mM Tris-HCl, pH 8.2, 10 mM EDTA, pH 7.2), made to 0.5% with SDS and mixed with 5 ml of phenol-chloroform (1:1, v/v)¹⁷. The mixture was homogenised for 2 min in a Teflon motor driven homogeniser and centrifuged at 23,000g for 10 min. The aqueous phase was removed and kept on ice, while the phenol phase and the interphase were re-extracted with 3.0 ml of extraction solution at 50 °C. After centrifugation, the aqueous phase and the interphase were combined with the previous aqueous phase, and re-extracted with 5 ml of phenol-chloroform for 10 min on a rotary shaker at room temperature. After centrifugation, the interphase was discarded and the aqueous phase was extracted again with 5 ml of phenol-chloroform. The aqueous phase was then mixed with 2 volumes of ethanol and kept overnight at -20 °C. The precipitate was washed twice with 3.0 ml of 3.3 M sodium acetate, pH 6.0 (ref. 18), once with 70% ethanol, 0.1 M potassium acetate, vacuum dried and dissolved in 50 mM potassium acetate, 10 mM Tris-HCl, pH 7.6. Aliquots, as indicated, were taken for determination of $^3\text{H-poly(U)}$ hybridisation¹⁹. The yields of total RNA were 2.5 mg per sample and were similar for both the dry and imbibed samples. The data for poly(U) hybridisation are corrected for a background of 1 ng (22 c.p.m.) hybridised in the absence of exogenous RNA.

lation of preformed mRNA to occur in early germination but it would not be obligatory to the recruitment of the preformed mRNA. In either event, polyadenylation is clearly not a prerequisite for polynibosome formation. Finally, as a direct test for the occurrence of polyadenylation, we have analysed total RNA of both dry embryos and those that had imbibed for 40 min. Table 3 shows that the capacity of the RNAs to hybridise $^3\text{H-poly(U)}$ were quite similar. Since the RNA content of both types of embryo was comparable (see legend to Table 3) we conclude that the poly(A) content of embryos either dry or after imbibition is similar, and that the quantity of newly synthesised poly(A)-containing mRNA is negligible in comparison with that pre-existent in the embryo. The mechanism by which the preformed mRNA is recruited into the translational system remains to be investigated.

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Fluoride concentrations in developing enamel

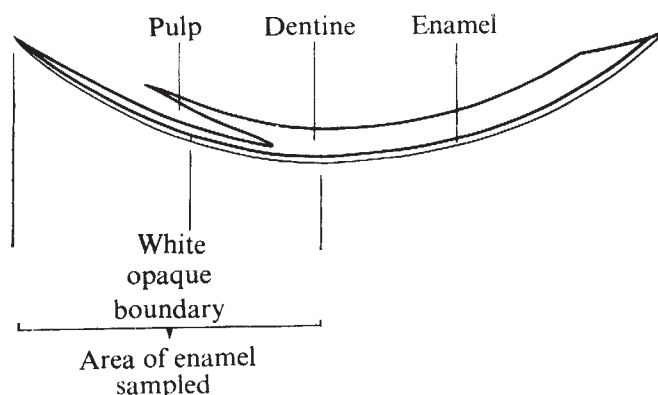
THERE is at present great interest in the effects of fluoride on the skeletal and dental tissues, owing to its considerable beneficial effect on dental health¹, its potential use in the treatment of bone disease^{2,3} and, somewhat paradoxically, its capacity to cause dental and skeletal fluorosis^{4,5}.

The effect of fluoride on teeth has been well documented and particular attention has been paid to its distribution in dental enamel. The relatively high concentration of fluoride in the enamel surface^{6,7} has been regarded by many as a protective barrier against carious attack. Considerable amounts of fluoride are present in the enamel before the tooth erupts into the mouth⁸ and are probably acquired during the period of tooth development when, as indicated by ^{18}F studies^{9,10}, fluoride ions are rapidly incorporated by the mineralising tissue. Fluoride exerts its effect on the caries susceptibility of the teeth¹¹ particularly during this period and it is only during the period of development that fluoride can bring about the changes associated with dental fluorosis¹². In spite of the obvious concern with this period of development, because of technical difficulties with microsampling and analysis, little or nothing is known about the concentrations of fluoride in developing enamel or in any other calcified tissue during its period of mineralisation.

Using recently developed micro-analytical techniques¹³ we therefore attempted to determine the pattern of fluoride distribution in the developing enamel of the continuously growing rat incisor; this was chosen because all stages of mineralisation can be seen in a single tooth. A preliminary study¹⁴ indicated that in normal animals the concentration of fluoride in the forming enamel decreased as the tissue matured. This was somewhat surprising in view of the many statements in the literature that fluoride concentrations in mineralised bone, dentine and enamel tend to increase with time¹⁵⁻¹⁷.

Subsequent work has confirmed our preliminary finding. Wistar rats reared on a stock diet (Oxoid rat cake, fluoride concentration 10-14 p.p.m. per dry weight) were killed at about 250 g body weight, the lower incisors removed and a series of about 20 enamel particles weighing 10-100 μg dissected from each tooth. These particles of enamel formed a series representing the different stages of development from partially mineralised tissue near to the root apex to the highly mineralised enamel of the maturing region (Fig. 1)¹⁸. Each enamel specimen was ashed and analysed for fluoride, using the Orion electrode¹⁹, and for phosphorus, using the method of Chen, Toribara and Warner²⁰. Figure 2 shows the variations in fluoride con-

Fig. 1 Diagram of rat incisor, indicating different stages of enamel development and regions from which enamel samples were taken.



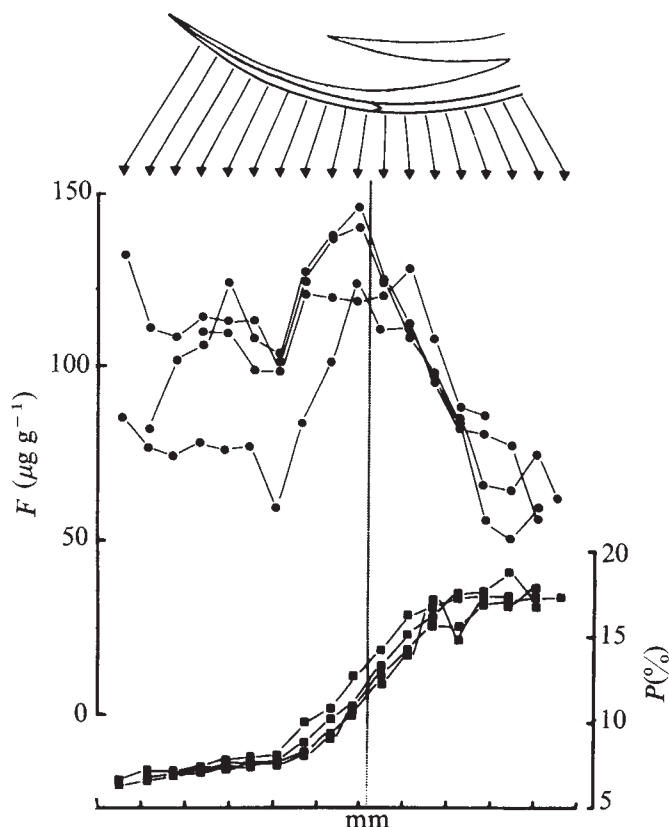


Fig. 2 Distribution of fluoride and phosphorus along the developing enamel of the continuously growing rat incisor (dry weight). The part of the tooth shown and analysed corresponds to the developing part of the root as indicated on the left-hand side of Fig. 1.

centrations and phosphorus concentrations in the developing rat enamel per dry weight tissue. The fluoride concentration was higher in partially mineralised than in the more highly mineralised maturing enamel. The concentration in the partially mineralised enamel was not constant, however. At or just before the stage of development in which the enamel began to mineralise rapidly, fluoride concentrations were particularly high. The concentration fell rapidly as the mineral content of the tissue approached that of mature enamel.

Since information about teeth of limited growth would be more directly relevant to the situation in the human dentition, we carried out similar determinations on enamel dissected from the developing incisors of bovine foetuses. The pattern of fluoride distribution along the enamel of each individual tooth was considerably different from that of the rat incisor. This was to be expected, since the enamel of each bovine tooth was at a different stage of development, depending on foetal age, and no single tooth exhibited the full spectrum of mineralisation equivalent to that of the rat incisor. A more complete developmental series must be examined before the full picture emerges but it seems from the teeth illustrated by Fig. 3a–d that, again, the fluoride content of the fully mineralised enamel is lower than that of the partially mineralised tissue.

Independently, R. L. Speirs has now also reported unpublished findings that the fluoride content of developing enamel from pig teeth decreases as the density of the enamel increases.

There are several possible explanations for this decrease in fluoride concentration. The first is that fluoride, perhaps acquired by ionic exchange when the enamel is only partially mineralised, might be diluted by the subsequent deposition of relatively fluoride-free enamel. This is

suggested by the rat data in Fig. 2. A similar suggestion was made by Gedalia, Garti and Epstein²¹ to explain an observed decrease in the fluoride concentration of developing human teeth between the eighth and ninth months of foetal life. The observed increase in mineral content of the foetal teeth, however, was insufficient to account fully for the decrease in fluoride concentration. In the bovine teeth (Fig. 3) a comparison between the decrease in fluoride concentrations and the concomitant variations in phosphorus content leads also to the conclusion that, here, dilution could not alone explain the fall in fluoride concentration.

Ruzicka and Mrklas (personal communication) suggested that since the fluoride concentration of the skeleton would increase with age, more skeletal fluoride would be released during the normal processes of bone remodelling and the fluoride concentration of the blood would gradually rise with age. This would in turn be reflected in the fluoride concentration of the most recently formed enamel, towards the root apex. Since Ruzicka and Mrklas made this interesting suggestion, it has been shown that the concentration of fluoride in blood does indeed reflect that in the skeleton²². Whether this could entirely account for such elevated fluoride concentrations in forming enamel, however, seems doubtful, for the change in the fluoride content of the skeleton over the period of incisor formation would be relatively small, and it would not alone explain the peak concentration of fluoride in the rat incisor just before the final phase of mineralisation; this peak will be mentioned later.

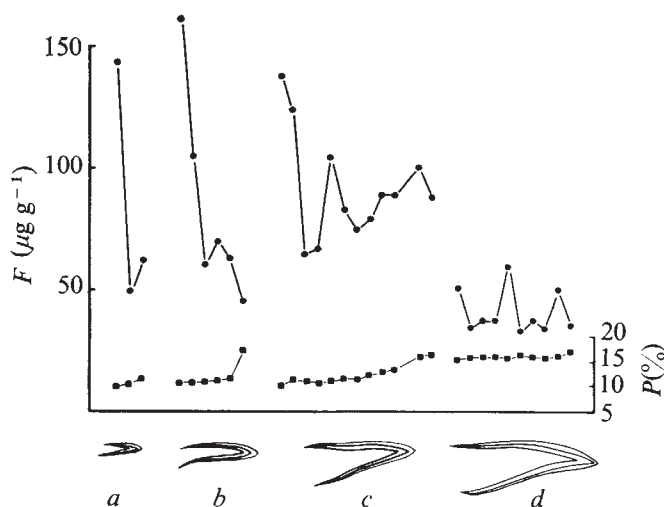


Fig. 3 Distribution of fluoride and phosphorus along the developing enamel of four deciduous bovine incisors taken from foetal calves aged; a, 6.0; b, 6.8; c, 7.8; and d, 8.7 month (dry weight).

The final possibility, and one which seems to have the greatest experimental support at present, is that some of the fluoride acquired during the early stages of enamel development is lost as the tissue mineralises. The ¹⁸F studies of Wallace-Durbin⁹ showed that the ¹⁸F activity of bones and teeth from rats injected with the isotope reached a maximum value and then decreased with time, suggesting that at least some of the fluoride acquired by the mineralising tissue may be lost again. A preliminary attempt by ourselves to relate the fluoride content of the enamel to the volume of the particles dissected from the rat incisor (a procedure intended to eliminate the possibility that the fluoride concentrations fell as a result merely of dilution by the increasing weight of mineral) also supported the view that some of the fluoride acquired during the earlier stages of development is lost as the tissue matures²³.

The recent unpublished findings of Speirs similarly point to fluoride loss.

Although the partially mineralised enamel of the rat incisor had, overall, a higher fluoride concentration than the maturing tissue, there was within this region a fairly consistent pattern of fluoride distribution. A peak of relatively high fluoride concentration usually occurred just before the phase of rapid mineralisation, that is, at or before the boundary between the translucent and opaque enamel, indicated by a V in Fig. 2. The position of this site seems to correspond to a region where ^{32}P -labelled phosphate is preferentially taken up both *in vivo* and *in vitro*¹⁸. The high ^{32}P activity found in this region seemed to reflect the physicochemical state of the enamel and perhaps fluoride, like phosphate, is incorporated into forming enamel mainly by diffusion into relatively permeable tissue. We have no information about the nature of fluoride binding in this highly hydrated region of tissue. The implication in the literature that fluoride is largely bound to the mineral part of enamel is almost certainly justified in the case of mature enamel but is perhaps not without question in this partially mineralised tissue. It seems possible that some of the fluoride might be free, or bound to the organic matrix, as suggested previously by Hammarström¹⁹, and perhaps subsequently lost as part of the matrix is withdrawn from the enamel during mineralisation.

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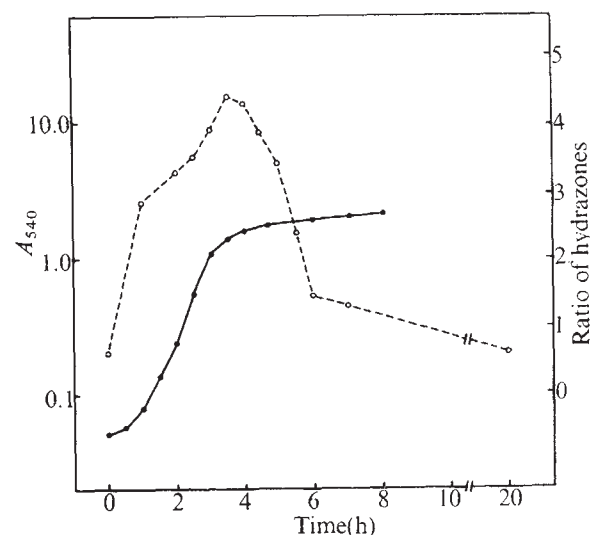


Fig 1. Growth-dependent changes in proportion of *Ps. aeruginosa* 16S rRNA molecules containing a 3'-terminal adenosine residue. *Ps. aeruginosa* was grown in L broth (1% bactotryptone, 0.2% yeast extract, 0.5% glucose, 0.5% NaCl)* at 37 °C with vigorous shaking. Samples were taken at intervals, the A_{540} determined, the RNA extracted* and the individual rRNA species labelled with ^3H -isoniazid^{7,9}. The proportion of 16S rRNA molecules terminating in either adenosine or uridine was determined by paper electrophoresis of the labelled nucleoside hydrazones released by pancreatic RNase digestion^{4,7}. ●, A_{540} ; ○, ratio of c.p.m. in adenosine-isoniazid hydrazone to c.p.m. in uridine-isoniazid hydrazone.

This hypothesis is based on two sets of observations. First, the degree of complementarity between the ribosome binding sites of coliphage mRNAs and the pyrimidine-rich 3' sequence of a number of bacterial 16S rRNAs reflects the cistron selectivity of the ribosome⁵. Second, the 3'-terminal trinucleotide of *Escherichia coli* 16S rRNA (UUA_{OH}) is the only trinucleotide able to recognise, by standard base pairing, all three nonsense triplets; the strengths of the proposed pairing interactions correlate with the different degrees of suppressibility of the three nonsense codons in suppressing strains of *E. coli*⁴. A similar role has previously been proposed for the highly conserved 3' terminus of eukaryotic 18S rRNA which also terminates in UUA_{OH} (refs 6 and 7).

3'-sequence analysis of rRNA from bacteria other than *E. coli* shows that the 16S rRNA of several species also terminates in UUA_{OH} (ref. 5). 16S rRNA from one member of this group, *Pseudomonas aeruginosa*, however, has a large proportion of molecules which lack the 3'-terminal adenylic acid and two 3' sequences, CUCUCCUUA_{OH} and CUCUCCUUA_{OH}, are found in approximately equal proportions; no such heterogeneity is found at the 3' termini of 23S rRNA from any bacteria examined (ref. 5 and J. S., unpublished).

As the proportion of 16S rRNA molecules lacking the 3'-terminal adenylic acid varied between different preparations of *Ps. aeruginosa* RNA, we have investigated the influence of bacterial growth conditions on 3'-terminal heterogeneity. In this report we show that the level of 3'-terminal adenylation of *Ps. aeruginosa* 16S rRNA is a function of bacterial growth rate.

Ps. aeruginosa, adapted to L broth⁸, was grown at 37 °C with vigorous shaking. Samples were removed at intervals, the cells collected by centrifugation and immediately extracted with phenol-cresol-aminosalicylate⁹. rRNAs (16S and 23S) were separated on sucrose gradients; in all instances the A_{260} ratio of 23S:16S RNA was 2:1. The 3' termini were labelled with ^3H -isoniazid and the RNA digested with pancreatic ribonuclease^{4,7}. The relative amounts of 3'-terminal adenosine and 3'-terminal uridine in 16S rRNA were determined by paper electrophoresis of the labelled digest^{4,7}. The

Growth-dependent changes in terminal heterogeneity involving 3'-adenylate of bacterial 16S ribosomal RNA

THE 3' terminus of the 16S RNA from the small subunit of bacterial ribosomes is directly involved in ribosomal function¹⁻³. On the basis of 3'-terminal sequence analysis of bacterial rRNAs, we have proposed that a short sequence of nucleotides in this region has a direct base-pairing role in both the initiation and termination of protein synthesis^{4,5}.

3'-terminal nucleosides of 23S rRNA were determined by the same method.

Figure 1 shows the proportion of 16S rRNA molecules containing 3'-terminal adenosine, plotted as a function of cell density. The 3'-terminal sequences of the 16S rRNA are initially UUA_{OH} and UU_{OH} in the ratio 0.6:1. By 1 h after inoculation, this ratio had increased to 2.8:1, continued to increase during exponential growth and peaked at approximately 4.5:1 in late logarithmic phase. As the cell growth rate slows the ratio rapidly declines to 1.5:1 in early stationary phase; the rapidity of this change suggests that 3'-adenylate residues are removed from pre-existing 16S rRNA molecules during this period. This ratio falls to 0.6:1 after overnight growth. No significant 3'-terminal heterogeneity was found in 23S rRNA isolated from the same samples; at all stages of the growth curve at least 90% of the 23S molecules terminated in PyCA_{OH}.

These results suggest that single adenylate residues may be added post-transcriptionally to the 3' terminus of 16S rRNA *in situ*. Terminal heterogeneity involving adenylic acid has also been observed at the 3' terminus of bacteriophage T₇ mRNAs cleaved from a large precursor RNA *in vivo*¹⁰. The same RNA species, when produced by specific *in vitro* cleavage of the T₇ precursor RNA with RNaseIII, contain no detectable 3'-terminal adenosine, suggesting that the 3'-adenylate residues are added post-transcriptionally¹¹. Note that these T₇ mRNAs also contain a 3'-terminal pyrimidine-rich sequence as is found in 16S rRNA (refs 5, 10 and 11). With T₇ mRNAs, the terminal addition of adenylic acid seems to be less specifically regulated than that reported here for 16S rRNA, as between one and three adenylate residues are added to the 3' terminus of the RNA (ref. 10).

In the light of these results, it is of interest that a number of bacteria (including *E. coli* and *Pseudomonas*) and also yeast, contain a ribosome-bound ATP-RNA adenylyltransferase¹²⁻¹⁷. *In vitro* this enzyme catalyses the 3'-terminal addition of adenylate residues to RNA and, at least for *Pseudomonas*, demonstrates a preference for the 3' end of rRNA, particularly 16S rRNA, over that of tRNA or homopolyribonucleotides¹⁵. In yeast, the amount of ribosome-bound enzyme decreases towards late log phase¹⁷.

We have also found substantial 3'-terminal heterogeneity in *Bacillus stearothermophilus* 16S rRNA (ref. 5) and low level heterogeneity at the 3' end of both *B. subtilis* and *Caulobacter crescentus* 16S rRNA. A small proportion (~12%) of *E. coli* 16S rRNA molecules (which normally terminate in UUA_{OH}) lack a 3'-terminal adenosine when isolated from early stationary phase cells or after a nutritional 'shift-down' (J. S., unpublished). There are previous reports of 3'-terminal heterogeneity in *E. coli* 16S rRNA (ref. 18).

The results presented here may reflect growth-dependent changes in the level or localisation of an enzyme responsible for the addition of 3'-terminal adenylate. Alternatively, changes in the degree of heterogeneity could arise from alterations in accessibility to adenylating enzymes of the 3' terminus of 16S rRNA in different functional states of the ribosome. In view of the suggestion that the 3' trinucleotide may recognise intercistronic nonsense triplets and terminator codons on mRNA (ref. 4), however, a direct regulatory role for the 3'-adenosine in converting UU_{OH} to UUA_{OH} (*E. coli*, *Ps. aeruginosa*), and CU_{OH} to CUA_{OH} (*B. stearothermophilus*, *C. crescentus*) should also be considered. Further studies on the functional activity of ribosomes containing 16S rRNA lacking a 3'-adenylate should resolve these questions.

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Differences in transcription of unique DNA sequences between neuronal and glial cells

OUR previous report¹ provided evidence for the existence of the brain-specific nuclear RNA using the RNA-DNA hybridisation method. As the neuronal and glial cells in the brain possess specific compositions and functions, each of these cells probably contains cell-specific RNA, as suggested by Church and Brown². In fact, Hydén *et al.*³ found remarkable differences in base composition between the total RNA of neuronal and glial cells. This difference may be accounted for by a greater proportion of cytoplasmic (ribosomal) RNA present in the neurone⁴. This report provides new evidence for the existence of cell-specific RNA in brain, using a unique DNA-³H-RNA hybridisation method.

Fifty minutes after injection of 5,6-³H-uridine (specific activity 43 Ci mmol⁻¹, Radiochemical Centre, Amersham) into the subarachnoidal space of a 15-18-d-old rat, the animal was decapitated. Then the brain was removed and homogenised in a solution containing 2.2 M sucrose and 1.5 mM CaCl₂, and the homogenate was centrifuged for 60 min at 70,000g. The precipitated nuclei were gently homogenised in 2.0 M sucrose containing 1.5 mM CaCl₂ and layered over a discontinuous gradient of 2.2, 2.35, 2.4 and 2.55 M sucrose. The rotor (Hitachi RPS 25-2) was spun for 120 min at 75,000g. The L fraction, recovered at the interface between 2.2 and 2.35 M sucrose, mainly contained neuronal nuclei; and the S fraction, obtained at the bottom, consisted of oligodendroglial nuclei. RNA was extracted from each cell nucleus with a SDS-hot phenol-bentonite procedure⁵ and treated with DNase (electrophoretically pure, Worthington, USA) and with Pronase, and finally passed through a Sephadex G50 column. Unlabelled RNA was extracted from the neuronal and glial nuclear fractions by a similar procedure. DNA was isolated from rat brain nuclei by using a modification of Marmur's method⁶. For RNA-DNA hybridisation with a DNA excess, DNA was sonicated to a molecular weight of about 170,000, as estimated by the method of Studier⁷, then precipitated with ethanol and further purified by filtration through Sephadex SP50.

To examine transcription in brain cells, two procedures were used: first, RNA-DNA hybridisation, using a membrane filter in the presence of formamide, and immobilisation of DNA on membrane filters (Millipore HAWP)⁸; and second, RNA-DNA hybridisation with excess DNA in solution^{9,10}. Figure 1a shows the results obtained with the membrane filter hybridisation technique. When the rapidly labelled RNA of the S fraction was competed with each unlabelled RNA of L and S fractions, almost identical competition curves were obtained with either RNA of the L or S fraction. In the case of labelled RNA of the L frac-

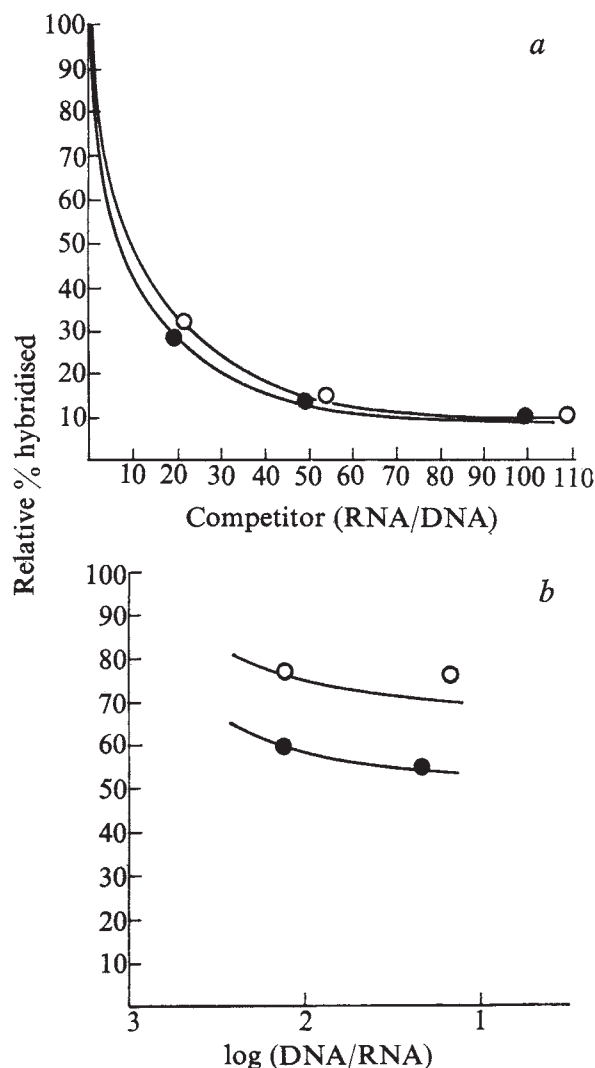


Fig. 1 Competition by unlabelled RNA from L fraction (○) and S fraction (●) in the hybridisation of ³H-labelled RNA from S fraction with brain DNA. *a*, RNA-DNA hybridisation was carried out at 35 °C for 25 h in glass vials in 2 ml 6-SSC-formamide (1:1 v/v). Each incubation medium contained 25 μg ³H-labelled RNA from S fraction (280 c.p.m. μg⁻¹), DNA filter (10 μg) and various amounts of unlabelled competitor RNA. Then the hybrid-containing membrane filters were incubated with RNase and washed on each side. Finally the filters were dried and counted in a scintillation counter. The radioactivity bound to DNA without competitor was 200 c.p.m. and is taken as 100%. *b*, Hybridisation with a DNA excess was carried out by a modified method of Melli *et al.*⁹. DNA fragments in 0.03 M phosphate-buffered saline (PBS) were heated at 100 °C for 10 min. ³H-labelled RNA was added to a single-stranded DNA solution. Reaction mixtures (1 ml) contained 3 mg DNA, 2.94 μg ³H-labelled RNA (1,660 c.p.m.) and unlabelled competitor RNA. The reaction mixture was placed in a water bath at 70 °C and the salt concentration was adjusted to 0.4 M PBS. At *C*₀t 10,000, the incubation reaction was stopped by removing the glass tube and placing it into an ice bath. The hybridisation mixture was diluted with water to a concentration of 0.24 M PBS, and treated with RNase (50 μg ml⁻¹) at 37 °C for 30 min. After RNase treatment the reaction mixture was precipitated with cold 10% TCA. The precipitate was collected on Whatman GF/C glass filter, washed with adequate volumes of 5% TCA and with ethanol, dried and counted in a scintillation counter.

tion, similar results were obtained. As the hybridisation conditions used in these experiments are known to detect differences in the transcription of repeated DNA sequences, the above results may indicate negligible differences in the transcription of the repeated DNA sequences between the L and S fractions.

When the reaction mixture was incubated to *C*₀t 10,000 at 70 °C with a DNA excess, about 40–50% hybridisation was reached in the absence of an unlabelled competitor.

As shown in Fig. 1*b*, when the rapidly labelled RNA of the S fraction was competed with each unlabelled RNA of the L and S fractions, the unlabelled RNA of the S fraction was much more effective in competition than was the L fraction. These results are indicative of significant differences in the transcription of unique DNA sequences between the two cell types. These differences are statistically significant ($P < 0.05$). Therefore, it can be concluded that the neuronal and glial nuclei have the cell-specific RNA species. The proportion of RNA transcribed from repeated DNA sequences seems to be only a minor fraction of the rapidly labelled nuclear RNA and much of this nuclear RNA is transcribed from unique DNA sequences. As some parts of these latter RNA species pass to the cytoplasm as mRNA, cytoplasmic mRNA should be the product transcribed from unique DNA (refs 9 and 11–13). Further, these premessenger nuclear RNAs contain polyadenylate sequences^{13,14}. Our present results may indicate the differences in such RNA species between two cell types. On the other hand, no differences are found in RNA transcripts from repeated DNA sequences which seem to be a minor fraction of the heterogeneous nuclear RNA with a small amount of premessenger RNA as described above. The function of such RNA transcribed from repeated DNA is not yet fully elucidated, although the hypothesis of Britten¹⁵ is interesting. As the cytogenetical relationship between two cell types is close¹⁶, it may be difficult to find the differences in such RNA using a membrane filter technique.

Recent reports on the saturation hybridisation experiment have shown that about 8% of mouse unique ³H-DNA is complementary to total brain RNA and that the complexity of the spectrum of RNA from other tissues is about 2%. Church and Brown² have suggested that these results reflect the diversity of cell types found in the brain. Our present results may be in agreement with their suggestion. On the basis of the experiment with the total RNA of malignant neural tissues, Grouse *et al.*¹⁷ have reported that neuronal and glial cells exhibit roughly the same extent of transcriptional diversity. Their results may be related to the use of the saturation technique and the total quantities of respective tissues. In the context of our present results, it may be interesting that neurones contain the specific protein like 14, 3, 2 (ref. 18), and glia also the specific protein like S100 (ref. 18). It will be necessary to isolate and characterise these cell-specific RNAs.

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Excision of thymine dimers from specifically incised DNA by extracts of xeroderma pigmentosum cells

SINCE the initial observations on defective DNA repair in fibroblasts cultured from patients with xeroderma pigmentosum (XP)^{1,2}, a large number of cases have been collected and studied in laboratories around the world. The results of these studies indicate a surprising degree of complexity in the DNA repair defect(s) in this disease. At an early stage it became apparent that not all cell strains are totally defective in the excision repair of ultraviolet radiation damage *in vivo*, there being varying degrees of "leakiness"³⁻⁵. Cell fusion studies have since indicated that a number of distinct genetic complementation groups exist, suggesting a multigenic basis for the disease⁶⁻⁹. In addition, defects in photoreactivation¹⁰ and in post-replication repair¹¹ of ultraviolet radiation damage in some XP strains have been reported.

If we confine our attention to those strains of XP cells that exhibit defects in excision-repair (ignoring for this study the variants which have normal excision repair^{12,13} but defective post-replication repair¹¹) then five distinct complementation groups, designated A,B,C,D,E, have been identified^{6,7}. Only one patient in group B is currently known.

At present, little is known about the biochemical basis of the repair defectiveness of XP cells in culture, or of the various complementation groups. In all cases specifically examined, indirect studies suggest the presence of an endonuclease or endonuclease-related defect. The evidence on which this suggestion has been made includes the ability of XP cells to repair DNA strand breaks from ionising radiation^{14,15} and alkylating agents^{16,17}, the failure of XP cells to repair damage to DNA bases that require endonucleolytic incision¹⁷⁻¹⁹ and the failure of XP cells to excise dimers^{2,20} or remove ultraviolet endonuclease-sensitive sites²¹ from their DNA. Direct demonstration of a defect in the incision step of repair by measurement of single strand breaks in DNA has not been achieved^{22,23}.

Since many of these studies were carried out in different laboratories, before complementation analysis began, it is not possible to state whether an apparent incision defect is characteristic of all complementation groups, but no exceptions have been reported except for the XP variant. Current understanding of the molecular mechanism of excision repair based on prokaryote models, calls for an endonucleolytic incision of DNA, excision of the damaged segment and repair synthesis. One might therefore expect that some cases of XP would show defects in the latter two steps. Nonetheless, the finding of a DNA incision defect in more than one complementation group is not inconsistent with the existence of multiple alleles, if the human cell ultraviolet endonuclease has multiple subunits and/or if other gene products are required for the endonuclease to function in human cells.

An alternative hypothesis first suggested by Haynes²⁴ is that the repair enzymes may function as a complex in a coordinated way, such that a defect in any one step would block the entire repair process. If this hypothesis were correct, then cases of XP defective in an excision nuclease or a DNA polymerase activity would still show a defect in the incision of ultraviolet-irradiated DNA and *in vitro* none of the enzymatic steps associated with excision repair should be demonstrable. On the basis of this kind of model, it has been suggested that all the data currently available on XP cells is consistent with either a defective endonuclease or exonuclease²⁵.

To obtain direct evidence for the validity of the enzyme complex hypothesis and further information on the repair

Table 1 Characteristics of XP cell lines used for dimer excision ability

Cell line	Neurological symptoms	Level of unscheduled synthesis*	Complementation group
XP 12 (SV40)	yes	0	A†
XP 25 RO	yes	0	A†
XP1 PW	no	4-7%	C‡
XP2 NE	yes	33-51%	D‡
XP 21 SF	no	60-65%	E‡

*Measured by autoradiography of fibroblasts labelled for 3 h with ³H-thymidine (10 μ Ci ml⁻¹ 15.8 Ci mmol⁻¹) after irradiation with 50-100 erg mm⁻².

†Assigned by complementation experiments^{6,8}.

‡Assigned by comparison of clinical symptoms of neurological involvement and levels of unscheduled synthesis with the groups described elsewhere^{6,7}.

defects in XP cells, we have developed a cell-free system that excises pyrimidine dimers *in vitro*²⁶. In our initial investigations^{26,27} we reported the presence of an exonuclease activity in extracts of normal human cells in culture, which excises thymine dimers from ultraviolet-irradiated DNA previously incised with a dimer-specific endonuclease purified from bacteriophage T4-infected *E. coli*²⁸. This activity excises thymine dimers in the 5'→3' direction, is stable to freezing, has a requirement for Mg²⁺ or Mn²⁺ and is sensitive to inhibition by SH-blocking agents. A similar activity has recently been purified from human placenta²⁹. We have now examined extracts of XP cells for dimer excising activity on previously incised DNA from four of the complementation groups discussed above. Fig. 1 shows the results obtained with extracts of a human cell line (HeLa) and an XP cell line (XP 12 [SV40] RO). Table 2 shows the excision of thymine dimers by extracts of a normal and four XP fibroblast strains. The results of a number of such experiments indicate no significant differences in the specific activity of excision nuclease in any of the XP classes compared with normal cells.

These results lead us to conclude, first, that the hypothesis of a coordinated enzyme complex for performing excision repair is not applicable to excision of pyrimidine dimers *in vitro*: extracts of all XP classes can excise pyrimidine dimers from an exogenous DNA when the first step of repair is completed by the T4 phage coded endonuclease. Second, there seem to be no examples of XP cells defective in the excision step of excision repair *in vitro*. This lends further support to the observation that the biochemical defects in excision repair in this disease may be related to events pertaining to endonucleolytic incision of damaged DNA.

We offer these conclusions with the following reserva-

Fig. 1 Details of the experimental procedures are described in the legend to Table 2. Each incubation contained crude protein in the amount indicated. ○, ×P 12 (SV) RO; ●, HeLa.

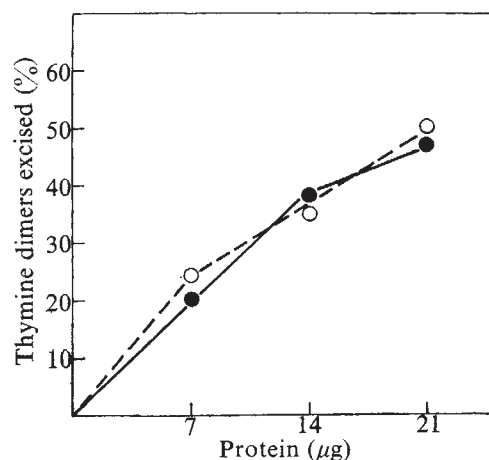


Table 2 Thymine dimer excision activity in normal and XP cell extracts

Source extract	Complementation group	Thymine dimers excised in 30 min (%)	Total acid-soluble nucleotides (%)
197 P (normal)	—	22	5.4
XP 25 RO	A	20	3.4
XP 1 PW	C	19	4.8
XP 2 NE	D	32	2.8
XP 21 SF	E	25	5.2

XP cells were obtained by skin punch biopsy and the normal fibroblast strain (197P) was obtained from foreskin. The cells were grown in minimal Eagles medium with 15% foetal calf serum (Gibco, Grand Island, NY) and 100 U ml⁻¹ of penicillin and streptomycin at 37 °C in a 5% CO₂ atmosphere. Cells were collected with trypsin-EDTA and washed three times with buffered saline. Cell cultures were checked for mycoplasma by the method of Todaro *et al.*³⁰ and showed no contamination. Cell-free extracts (microscopic examination revealed complete disruption of cells and nuclei) were made in 0.15 M NaCl, 0.01 M Tris-HCl buffer pH 8.0 and 0.005 M β-mercaptoethanol with 30 strokes of a Dounce homogeniser using a tight-fitting pestle. Incubation mixtures (0.1 ml) contained 22.5 nmol of ³H-*E. coli* DNA previously ultraviolet-irradiated at a fluence of 334 J m⁻² and incised with T4 ultraviolet endonuclease²⁸. This DNA was shown to have endonucleolytic incisions on the 5' side of 80–95% of the thymine dimer sites. Also present in the mixture were MgCl₂, 0.1 M; Tris-HCl buffer, pH 8.0, 0.01 M and 140 μg ml⁻¹ protein from the crude extract. Incubation was at 37 °C for 30 min. Reactions were terminated by the addition of 0.1 ml 10% cold trichloroacetic acid. The acid-insoluble fractions were hydrolysed and thymine dimer contents determined as described previously³¹.

tions in mind. First, these studies have used cell-free preparations and we have no direct evidence that the enzyme activity or activities measured, function in dimer excision *in vivo*. Second, the substrate used consists of ultraviolet-irradiated *E. coli* DNA incised with an endonuclease purified from phage-infected cells. It is possible that the human UV endonuclease acts in a manner which precludes the action of the nuclease activity we have measured and creates substrate sites for a different enzyme. It is also possible that the excision step involves an additional factor(s) in order to accommodate the complexity of the chromatin substrate in living cells.

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Regeneration of the photoprotein aequorin

THE photoprotein aequorin (molecular weight about 30,000) isolated from the bioluminescent jellyfish *Aequorea aequorea*^{1–3} emits blue light ($\lambda_{\max}$ 470 nm)⁴ by an intramolecular reaction in aqueous solution when Ca²⁺ is added, in the presence or absence of molecular oxygen¹. This reaction has been postulated⁵ to follow the scheme in (Fig. 1), in which an essential component H₂O₂ of aequorin is bound to the protein part in a form such as a peroxyacid or an α-hydroxyhydroperoxide which readily releases H₂O₂. Hypothesised roles for peroxide in aequorin (see recent review⁶) are consistent with this scheme. YC is a yellow chromophore of unknown structure, responsible for the colour of aequorin from which it has been isolated⁶, and found to be different from other components in Fig. 1.

The luminescence reaction requires the binding of three Ca²⁺ to one molecule of aequorin⁴. The product is a blue fluorescent protein (BFP) that exhibits a fluorescence spectrum exactly corresponding to the blue luminescence of the Ca²⁺ triggered reaction⁴. In BFP, the light-emitting moiety (Fig. 1, compound I), which is not fluorescent itself in aqueous solution, binds to the protein part (dissociation constant 5×10^{-6} M)⁷ yielding the fluorescent BFP (ref. 8). We have now found that the protein moiety of spent aequorin reacts with compound II in the presence of molecular oxygen, leading to regeneration of aequorin (Fig. 1). Compound II, initially found in aequorin in its enolised structure⁵, is evidently the same as natural luciferin of *Renilla*⁹. We have named this compound coelenterazine⁹ in view of its widespread occurrence in luminescence systems of coelenterates^{8,9,10}.

A sample of electrophoretically pure aequorin^{3,4} was luminesced by addition of Ca²⁺, and the resulting BFP was passed through a column of Sephadex G25 (Pharmacia) prepared with 0.01 M Tris-HCl buffer (pH 7.5) containing 0.01 M EDTA, to separate the protein moiety and compound I. The former eluted in the void volume, whereas the latter was adsorbed on the column. Treatment of this protein with a large excess of synthetic compound II (ref. 11), in the presence of molecular oxygen plus 2 mM 2-mercaptoethanol and 5 mM EDTA, at 5 °C, gave rise to Ca²⁺-triggerable photoprotein. The yield of photoprotein was 50% after 25–30 min, and nearly 90% within 3 h, with reference to the amount of protein molecules. After 20 h the reaction mixture was passed through a column of Sephadex G25 (prepared as above) to separate photoprotein from reagents. The photoprotein thus obtained was identical to the original aequorin as evinced by absorption spectrum ($\lambda_{\max}$ 281 and 460 nm, $A_{460}/A_{281} = 0.030$), luminescence spectrum ($\lambda_{\max}$ 470 nm), luminescence activity (1.55×10^{15} photons ml⁻¹ at 25 °C when A_{280} is 1.0 cm⁻¹), kinetics of luminescence in the presence and absence of air (Fig. 2), and chromatographic behaviour on DEAE-cellulose and Sephadex G100. Regeneration is thus confirmed; it was not influenced by various concentrations of EDTA or by the addition of H₂O₂, but it did not take place in an evacuated reaction vessel without oxygen.

When compound II was added to a solution of separated

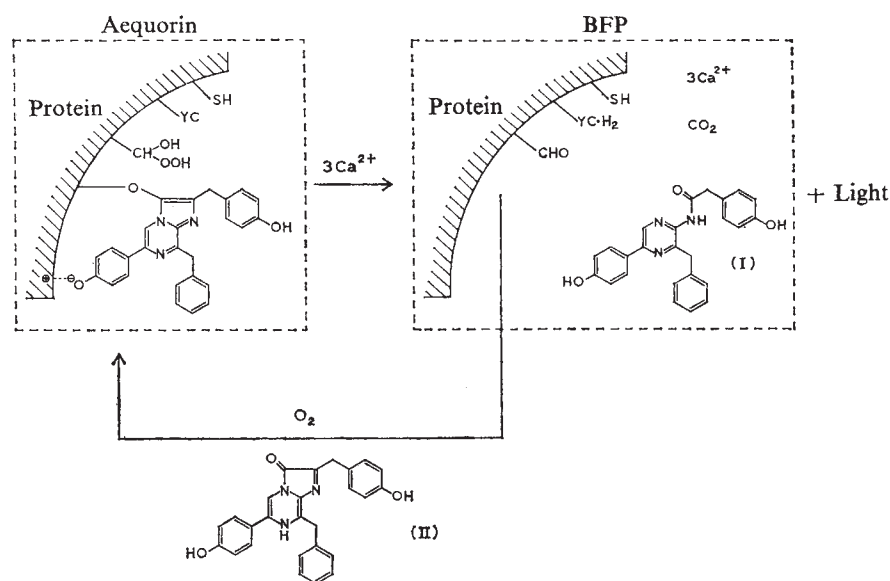


Fig. 1 Postulated mechanism of luminescence⁵ and regeneration of aequorin. Bound H_2O_2 in aequorin is tentatively shown as α -hydroxyhydroperoxide. After the luminescence reaction, YC in aequorin is reduced to a colourless form $\text{YC}\cdot\text{H}_2$, but the sulphydryl (SH) group(s) essential for light emission is ultimately unchanged. CO_2 in BFP may be bound to the protein.

protein moiety together with Ca^{2+} , or directly into spent aequorin, that is, into the solution of BFP containing Ca^{2+} , aequorin was regenerated and luminesced simultaneously as Ca^{2+} was already present. The result was thus a weak, continuous luminescence (Fig. 3). The rate of slow light emission in such circumstances obviously parallels the rate of regeneration of aequorin, in as much as the usual Ca^{2+} -triggered luminescence of aequorin is very fast (see Fig. 2). In the presence of 2-mercaptoethanol plus an excess of II, the weak luminescence continued for many hours, resulting in a total photon emission at least five times that of the fast luminescence of the aequorin originally used. In the absence of 2-mercaptoethanol, however, luminescence gradually died away, resulting in a total photon emission only 10–15% that which occurs in the presence of 2-mercaptoethanol. Thus, this reagent seems to protect the functional sulphydryl group(s) during regeneration.

According to the above, the protein moiety of spent aequorin

Fig. 2 Ca^{2+} -triggered luminescence of native aequorin (○) and regenerated aequorin (●). Curves for 10^{-2} (a), 10^{-4} (b) and 10^{-6} M Ca^{2+} (c) are offset at 1, 2, and 3 s, respectively, for 0 time. Luminescence was initiated by quick addition of 3 ml 10 mM Tris-HCl buffer (pH 7.2) containing calcium acetate to aequorin solutions consisting of 3 μl aequorin stock solution containing 0.2 mM EDTA (A_{280} 1.44 cm^{-1} for both native and regenerated aequorin) plus 0.3 ml water. To avoid contamination of the more dilute (10^{-4} , 10^{-6} M) Ca^{2+} solutions, plastic rather than glass containers and pipettes were used throughout, except for a Hamilton syringe to measure the aequorin stock solution. The absence of any effect due to the presence of oxygen is shown by data (▲) on luminescence of regenerated aequorin with 10^{-2} M Ca^{2+} in a vessel thoroughly evacuated by a vacuum pump. Temperature: 24 °C.

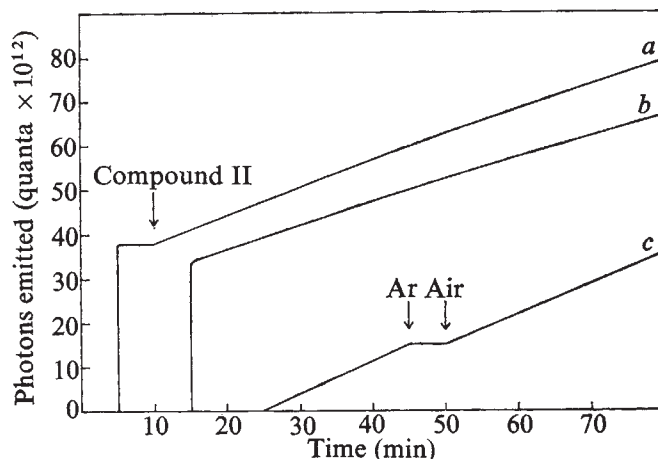
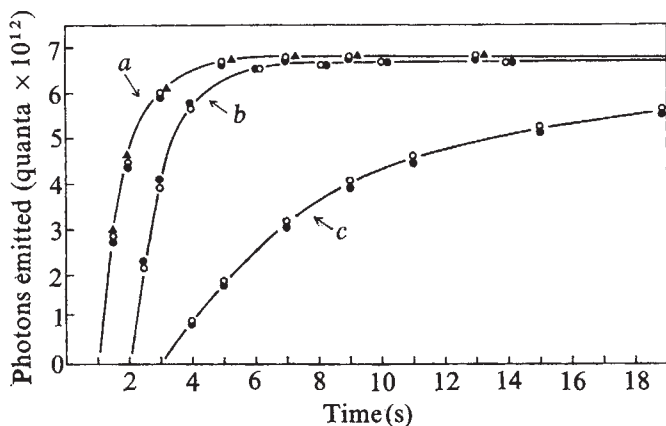


Fig. 3 Ca^{2+} -triggered luminescence of aequorin and regenerated aequorin: a, 4 ml 10 mM Ca acetate was added to 8.7 μg aequorin in 1 ml buffer at 5 min, followed by addition of 6 μg II in 20 μl methanol at 10 min; b, 8.7 μg protein moiety of BFP and 6 μg II were added to 1 ml buffer; the mixture was kept at 5 °C for 3 h, then placed in the light-measuring apparatus at 0 min, and 4 ml 10 mM Ca acetate was added to this mixture at 15 min; c, 4 ml 10 mM Ca acetate containing 6 μg II was added to 1 ml buffer containing protein moiety of BFP at 25 min; bubbling the mixture with Ar gas started at 45 min; Ar was switched to air at 50 min, then bubbling was stopped after 15 s. The buffer (pH 7.5) in all cases consisted of 10 mM Tris-HCl, 5 mM EDTA and 2 mM 2-mercaptoethanol. The amount of aequorin was estimated from A_{280} ($E1\%1\text{cm} = 27.0$) (ref. 2). The amount of protein moiety of BFP was calculated assuming that aequorin yielded the same amount of the protein moiety. All luminescence measurements were carried out at 20 °C. The quick flash at 5 min is caused by native aequorin; all other light emissions are caused by regenerated aequorin.

can be regarded as an enzyme catalysing a luminescent oxidation of coelenterazine (II). In this view, aequorin itself must be considered to represent an intermediate, as hypothetically suggested by several investigators⁸. If so, by its known properties it is an indissociable intermediate^{1,2} that is extraordinarily stable (in the absence of Ca^{2+}).

The extent to which regeneration takes place in the organism is uncertain, but the presence of coelenterazine enol-sulphate in the photogenic tissues of *Aequorea* in an amount equivalent to as much as 5% of the aequorin molecules⁹ suggests that the photoprotein recycles *in vivo* as well as *in vitro*. Terminal steps in the generation of aequorin *in vivo* possibly follow the same pathway as regeneration.

The above results are interesting also in regard to the use of

aequorin luminescence as a fast, sensitive test for presence of Ca^{2+} in biological systems¹²⁻¹⁹. Inclusion of coelenterazine in the test system should prolong considerably the luminescence life of aequorin.

A recent paper²⁰ contains statements which amount to the suggestion that YC of the present paper is in effect *Renilla* luciferin. We have shown, however, that *Renilla* luciferin is actually identical to coelenterazine⁹. It should be emphasised that the absorption spectrum of YC (ref. 5) is significantly different from that of coelenterazine except for the one peak in the visible region. Moreover, recent mass spectrometric data (O.S. and F.H.J., unpublished) have demonstrated that YC and coelenterazine are in fact different compounds.

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Erratum

In the article "Computer simulation of protein folding" by M. Levitt and A. Warshel (*Nature*, **253**, 694; 1975) the wrong version of Fig. 4 was printed. The correct version is reprinted below.

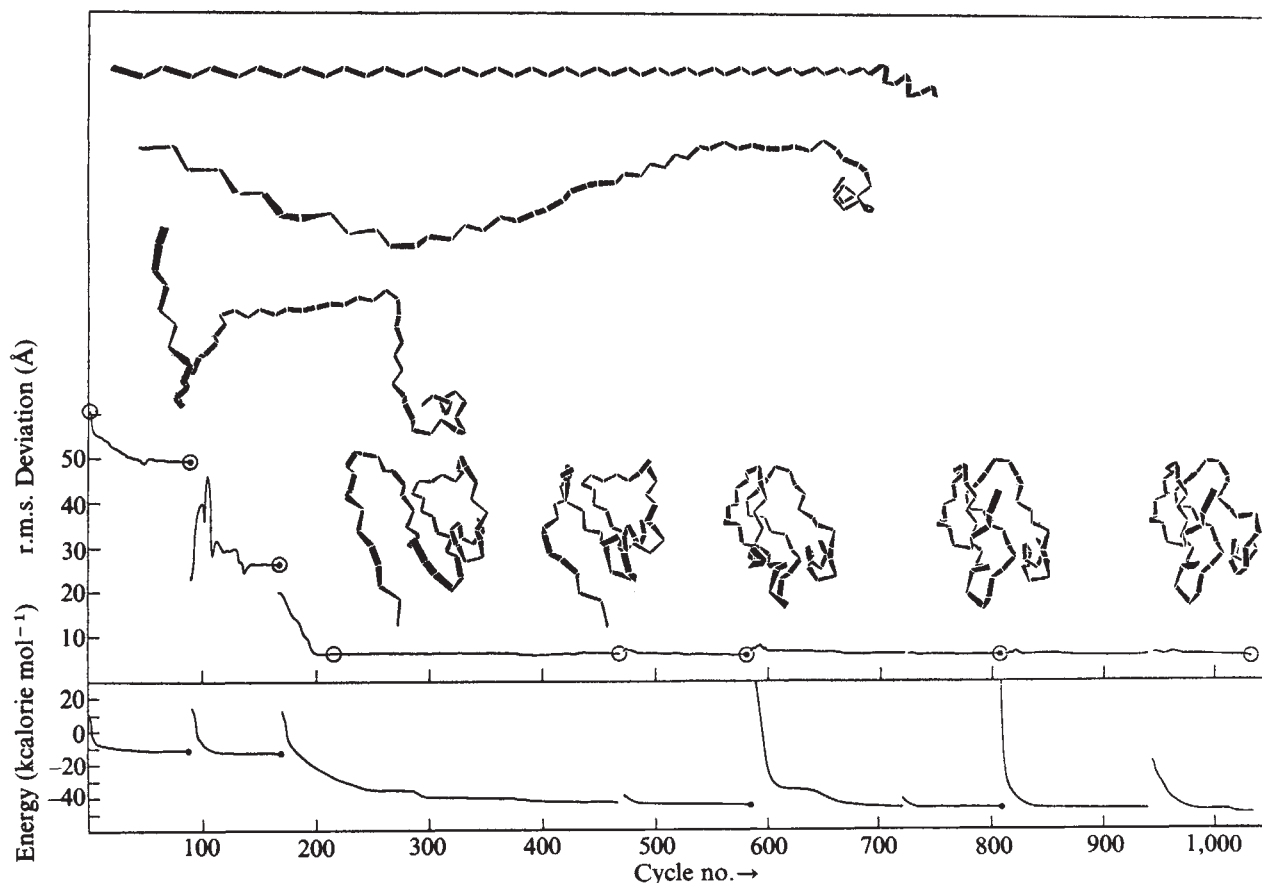


Fig. 4 Simulation of PTI folding from an extended starting conformation with the terminal helix ($\alpha = 180^\circ$ for all except 48 to 58 where $\alpha = 45^\circ$). No knowledge whatsoever about native PTI is used during this simulation (apart from setting the terminal helix). The conformation was thermalised at the end of each minimisation except near cycles 490 and 730 when the energy rises slightly because the minimisation was restarted after rounding the torsion angles to one degree. In the first two thermalisations, each normal mode was perturbed in the plus direction to raise the associated energy to $R(n)kT/2$ with $T = 1,000$ K. In the other three thermalisations, the perturbations were randomly in the plus and minus directions but always such as to raise the energy by $kT/2$ with $T = 300$ K. (Because the random numbers are distributed uniformly rather than exponentially, these temperatures do not correspond to the macroscopic temperature.) The 8 ribbon diagrams, which show the C α chain path, refer from left to right to the 8 conformations at the circled points on the r.m.s. deviation curve, respectively. The last five conformations have progressively lower energies and are each a little closer to the native structure ($E = -43.3, -45.7, -46.0, -46.9$ and -48.9 kcalorie mol $^{-1}$, respectively; r.m.s. deviation = 6.08, 5.7, 5.6, 5.4 and 5.3 Å, respectively). The solid dots at the end of a minimisation indicate that a perfect minimum was reached (r.m.s. gradient less than 10^{-6} kcalorie mol $^{-1}$ rad $^{-1}$). One cycle takes about 0.6 s on an IBM 370/165 computer.

matters arising

PCB concentrations in North Atlantic surface water

CONCENTRATIONS of polychlorobiphenyls (PCBs) have supposedly¹ declined 40-fold over wide regions of the North Atlantic from levels of about 30 ng l⁻¹ in 1971–72, to 0.8 ng l⁻¹ in 1973–74. This decline has been attributed¹ to the restriction of sales of PCB for non-captive uses during the period 1970–73, both in Europe and in North America. Much as we would like to believe these conclusions, they seem unfounded to us, and they seem to ignore the dynamics of the PCB-seawater system.

From estimates of PCB concentrations in 0.3 µm filtered seawater from 41 North Atlantic stations, Harvey *et al.* estimated a standing stock of dissolved PCB in the upper 200 m of the North Atlantic of 2 × 10⁴ tonnes (t) in 1972 (ref. 2) in addition to whatever quantity was bound up with <0.3 µm of particulate material; they suggested that the latter could increase the amount of PCB in the 1972 standing stock by 10%.

Using the reported 40-fold decline in the PCB concentration, and a model based on a simple exponential loss-rate we have derived a mean residence time, *r*, for PCB in seawater in the North Atlantic. Our model simulates the loss process on a daily basis by reducing the standing stock, *S*, by *S/r*. A range of values of *r* was tested to discover the value which reduced *S* (= 2 × 10⁴) by 97.5% (= 40-fold) at the end of 365 d. The model indicated a mean residence-time of just under 100 d. In order for such a system to sustain the reported standing stock of 2 × 10⁴ t, this value of *r* requires an annual input of 7.3 × 10⁴ t (representing the loss from the stock over 365 d).

That seems absurd in relation to what is known about the total production of PCB during the period 1960–71 (Table 1) because it suggests that a standing stock

results are not correct, so that the 1971–72 data (24–41 µg l⁻¹) are too high or the 1973–74 data (0.8–2.0 µg l⁻¹) are too low; or, second, that the extrapolation from individually correct data to a standing stock for the whole North Atlantic is at fault.

It seems relevant to note that three different analytical methods were used to obtain the two sets of data, that no evidence is presented on the repeatability of individual data and that, at least for the 1971–72 data, 36 of the 52 samples were collected with buckets at the water surface and so must have included an unknown and variable quantity of material from the surface microlayer which is known to be highly enriched with hydrocarbons even in the open ocean³.

Further, if the results from the 1971–72 analyses proved to be too high to be representative of water between the surface and a depth of 200 m over the North Atlantic as a whole, it would also resolve the paradox that has been evident for some years between those data and the relatively low values (about 1.0 ng l⁻¹) reported from coastal waters of the generally rather contaminated California Current⁴ and those (<10.0 ng l⁻¹) from the Irish Sea⁵, a region likely to be relatively highly contaminated.

The extrapolation from the two sets of data to two levels of standing stock for the whole North Atlantic (which is based simply on an estimate of the volume of the 0–200 m layer of that ocean) also seems to be not very well founded. The data are log-normally distributed, and so extrapolation would have been more appropriate from the geometric rather than the arithmetic mean; such a statistical distribution is likely to be a result of the very high degree of variability within regions (Harvey² reports a range of 1–150 µg l⁻¹ over a distance of only 80 km in the open Atlantic) or to the fact that both the

Quinn *et al.*³ are widely separated: in 1971 they came from a line between Newfoundland and Iceland, but in 1973 they came from the Sargasso Sea. We therefore suggest that the data and interpretations presented by Harvey *et al.*¹ are insufficient evidence on which to base the deduction that recent partial controls on uses of PCBs have already produced a dramatic decrease in the contamination of the upper layers of the open Atlantic Ocean.

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- ¹ Harvey, G. R., Steinhauer, W. G., and Miklas, H. P., *Nature*, **252**, 387 (1974).
- ² Harvey, G. R., Steinhauer, W. G., and Teal, J. M., *Science*, **180**, 643 (1973).
- ³ Bidleman, T. F., and Olney, C. E., *Science*, **183**, 516 (1974).
- ⁴ Goldberg, E., *NSF IDOE Baseline Conference*, 11 (National Science Foundation, Washington, DC, 1972).
- ⁵ *The Seabird Wreck in the Irish Sea, Autumn, 1969*, Natural Environment Research Council, Series C (4), 14 (1971).
- ⁶ *Polychlorinated Biphenyls in the Environment* (US Department of Commerce, Washington, DC, 1972).

HARVEY AND STEINHAUER REPLY—We disagree with Longhurst and Radford¹ that our analytical results are incorrect. After several comparisons of the different methods, analytical standards and reproducibility (± 20%) we are quite certain that our data are correct as they stand. It is relevant that during 1971–72, when about 30 ng l⁻¹ PCB was being measured we were also measuring 1 ng l⁻¹ or less in tapwater and groundwater. Also, in 1973–74 we measured PCB concentrations of 30–300 ng l⁻¹ in polluted estuaries near Boston, USA, at a time when North Atlantic values had declined to 0.8–2.0 ng l⁻¹. Clearly, the methods were capable of determining PCB concentrations present in different water masses over a wide range of values during the period in question.

We now agree, however, that our previous extrapolation was not justified. In the light of some recent data it seems that each area of the North Atlantic (the central gyre, doldrums, trades, and so on) is characterised by clusters of similar PCB concentrations which roughly correlate with salinity, wind direction and latitude². As we do not have data from north of 45° N since 1972, nor from south of 20° N before 1973, our extrapolation from an average concentration to the

Table 1 Global production (t × 10⁴) of PCB, assuming US production to be half of global production⁶

1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971
3.44	3.32	3.48	4.06	4.62	5.50	5.90	6.84	7.52	6.94	7.72	3.66

of the magnitude reported by Harvey *et al.* could only be achieved if the total world production of PCB were put continuously into the North Atlantic.

For a more reasonable explanation for this result, two possibilities should be considered: first, that the analytical

1971–72 and 1973–74 data comprise regional clusters of stations, each cluster being widely separated from others, so that there was little geographical correlation within or between the two sets of data. Even the two sets of data, one in each period, derived from the work of

entire North Atlantic was not warranted. In contrast, PCB concentrations have been measured regularly in the North American Basin during 1972–75. As this is the closest ocean basin downwind of the US industrial complex the large decrease observed there between 1972 and 1973 may only reflect changes in the North American PCB input and may have been the most rapid in the North Atlantic.

We intend to measure PCB concentrations north of 50°N during 1975 in waters more within the influence of European PCB losses.

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¹ Longhurst, A. R., and Radford, P. J., *Nature*, **256**, 239 (1975).

² Harvey, G. R., and Steinhauer, W. G., *Proc. second int. Conf. Environ. Biogeochem.* (in the press).

Avian vision

In an article¹ on the visual control of head movements by walking birds, Friedman quotes me as maintaining that a bird's head moves backwards as well as forwards during walking. This is not so. In 1931 observation of hens had shown me that, as Friedman has proved for pigeons, the head of a walking hen is thrust forwards as rapidly as possible and then remains stationary while the body of the bird catches up. It seemed to me quite clear that this enables the hen to have a succession of sharply defined pictures of its environment rather than the continuously slightly fuzzy picture which would be obtained from steadily moving eyes.

Humans circumvent this problem differently. They fix visually upon a succession of points for varying times according to their momentary interest, and use a reflex capacity to control eye positions to compensate for bodily movement.

Most birds have a much larger area of high definition than humans, backed by a much less versatile data processor, and may find it advantageous to attend only to those objects in their environment that are moving independently. These can be distinguished from a complex background only when the eyes are stationary with respect to that background and are not confused by the apparent backward displacement of the nearer objects which will occur as a result of parallax while the eyes are moving.

In my article² I was not concerned at all with the head movements of walking birds but with the frequent up and down movements of the heads of stationary birds. This, I suggested would not only give feeding birds

longer ranges of vision—which it by no means always does—but would enable birds to use parallax to obtain a three-dimensional picture of their surroundings.

Binocular stereopsis is available to most birds, with their sideways-looking eyes, only over a very narrow field of forward vision, and with an extremely short 'baseline' between the eyes. Observation of the changes of the retinal image as a bird raises and lowers its head, however, provides the bird with a much longer baseline and gives the possibility of three-dimensional vision over the whole field of view of both eyes—often nearly 360°.

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¹ Friedman, M. B., *Nature*, **255**, 67–69 (1975).

² Fremlin, J. H., *New Scientist*, **56**, 26–28 (1972).

Plume spacing and source

SINCE the average distance between island-arc volcanic centres (~70 km) is approximately equal to the depth to the magma source (~100 km), Lingenfelter and Schubert^{1,2} have suggested that the typical distance between hot spots (~3,000 km) is approximately equal to the depth to their source. Thus, they infer that the region of instability giving rise to the initially solid plumes of mantle material is near the core–mantle boundary. Certain fluid dynamic results may show this inference to be untenable.

In the case of island-arc volcanism³, andesitic magma is generated along the Benioff Zone at a depth of about 100 km. The magma is much less dense than the overlying peridotitic mantle material and this two layer system, andesitic magma beneath peridotitic mantle, is gravitationally unstable. This situation is analogous to that of a layer of oil underlying a layer of water, and represents a type of fluid instability commonly known as Rayleigh–Taylor instability^{4–6}.

When perturbed from equilibrium, the less dense, lower fluid penetrates the overlying fluid at discrete and, ideally, evenly spaced points. This is the case in both two and three dimensions^{4,5}. The characteristic wavelength of instability is dependent on the depth to the source layer (or thickness of the upper layer) only if the thickness of the source layer is greater than one tenth of the depth to the source⁷. That is, once the upper layer is 10 times thicker than the lower buoyant layer the dominant wavelength of instability is independent of the depth to the buoyant layer. In island arcs, judging from the

exceedingly small quantity of lava observed at the surface, the thickness of the magmatic layer is, surely, much less than 10 km. For those cases, the spacing of the volcanic centres is given by⁴

$$\lambda = (2\pi h_2/2.15)(\eta_1/\eta_2)^{1/3}, \text{ for } h_1 > 10h_2$$

where: λ = the dominant wavelength or spacing of volcanic centres; h_1 = the thickness of higher density layer; h_2 = the thickness of lower density layer; η_1 = the viscosity of denser layer (mantle); η_2 = the viscosity of lighter layer (andesitic magma).

By estimating roughly the contrast in viscosities ($\eta_1/\eta_2 = 10^9$), and from the observed spacing of the volcanic centres (~70 km), the magma layer in the case shown was probably a few tens of metres thick at the time of instability.

In the case of plumes, assuming, as did Lingenfelter and Schubert¹, that the process of instability is similar to that operative in island arcs, if the source layer is near the core–mantle boundary it must be greater than about 300 km thick for the plume spacing to reflect the depth to the source (that is, 3,000 km). Seismic evidence seems to indicate that a low density layer of this thickness cannot be present in the lowermost mantle. On the other hand, if it is assumed that the depth to the buoyant layer, wherever it is, is greater than 10 times the thickness of the buoyant layer itself, the thickness of that layer can be calculated from the relationship given already. For $\lambda = 3,000$ km and $\eta_1/\eta_2 = 10^9$, the buoyant layer thickness is about 100 km, whereas for $\eta_1/\eta_2 = 10^6$ it is about 10 km. A layer 10–100 km thick could be located seismically. Since the existence of a low density layer is a necessary condition for this type of fluid instability, which seems inherent in the plume theory as envisaged by Morgan, it is of paramount importance to ascertain its presence.

In conclusion, the distance between island-arc volcanic centres tells us little about the depth to the magma source and this is also probably true for the spacing between plumes if they arise from a Rayleigh–Taylor type fluid instability.

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¹ Lingenfelter, R. E., and Schubert, G., *Nature*, **249**, 820 (1974).

² Lingenfelter, R. E., and Schubert, G., *Bull. volcan.* (in the press).

³ Marsh, B. D., and Carmichael, I. S. E., *J. geophys. Res.*, **79**, 1196–1206 (1974).

⁴ Selig, F., *Geophysics*, **XXX**, 633 (1965).

⁵ Biot, M. A., *Geophysics*, **XXXI**, 53 (1966).

⁶ Whitehead, J. A., Jr., and Luther, D. S., *J. geophys. Res.*, **80**, 705–717 (1975).

⁷ Biot, M. A., and Odé, H., *Geophysics*, **XXX**, 213, (1965).

reviews

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LIND'S *Studies in Pre-Vesalian Anatomy** is a massive work; a stout quarto of more than 300 double-columned pages, which has been meticulously researched in libraries and archives in Europe and America. The fruits of Professor Lind's labours of the past 12 years are abundant. We are given 16th century documents—wills, letters, university and municipal records—which previously have never been published. We are given biobibliographies of eight pre-Vesalian anatomists. Finally, Lind provides elaborately annotated translations of six anatomical treatises originally published between 1497 and 1559. The result is a wonderfully full picture of the state of anatomy in the half century before Andreas Vesalius' *De humani corporis fabrica* (1543) transformed the subject. While Vesalius' genius is actually thrown into relief by comparison with his contemporaries, Lind's study documents the rich and vibrant anatomical tradition which preceded the *Fabrica*. Vesalius did not emerge from a vacuum.

With two exceptions, all of these pre-Vesalian anatomists were Italian, a fact which underscores the importance of the Italian universities in 16th century medicine. One of the exceptions was Andrés de Laguna (1499–1560), a peripatetic Spaniard who spent nine years in Italy. De Laguna wrote more than 30 books, including the *Anatomica methodus* (1535) which Lind has translated, though this is perhaps the least original of the texts which he has chosen. Though de Laguna himself was a figure of considerable fame, his *Anatomica methodus* was inferior to various similar works which also preceded Vesalius. One of these was Niccolò Massa's *Liber introductorius anatomiae* (written by 1536 though dated 1559 on the title page), also translated in the present volume. Educated at Padua, Massa (1499–1569) was an active physician who practised in his native city of Venice and who wrote widely on plague and syphilis. Whereas de Laguna returned solely to the ancients for authority, Massa was aware of the tradition of anatomical study in the west,

**Studies in Pre-Vesalian Anatomy: Biography, Translations, Documents*. (Memoirs of the American Philosophical Society, vol. 104.) By L. R. Lind. Pp. 344. (American Philosophical Society, 1975.) \$18.00.

Groundwork for a transformation

that had begun with Mundinus (1275?–1326). Mundinus was also revered by Berengario da Carpi (1460–1530), whose *Commentary on Mundinus* (1521) is summarised in this volume. Lind published an annotated translation of Berengario's more famous *Short Introduction to Anatomy* (the *Isagogae breves* of 1522) in 1959.

Berengario was one of a group of several outstanding anatomists who were prominent between the time of Mundinus and Vesalius: a group which included Gabriele Zerbi (1485–1569), Alessandro Achillini (1463–1512) and Alessandro Benedetti (1450?–1512). Lind has translated Achillini's *Annotationes anatomicae* (1520) and Benedetti's *Anatomice* (1502). Lind's work is rounded off with translations of Johannes Dryander's famous, short *Anatomia capitis humani* (1536) and Giovanni Battista Canano's equally familiar *Musculorum humani corporis picturata dissectio* (1541?). The well known illustrations of these latter two works are also reproduced.

From this summary the richness of Lind's work should be apparent. Lind provides the material necessary to any consideration of the development of anatomy between about 1500 and 1540. His annotations point back to the sources—classical, Arabic, and contemporary—of these pre-Vesalian anatomists. He notes the philosophical preoccupation of most of these figures, several of whom were equally at home in the world of 16th century Aristotelianism. In fact, part of Vesalius' suc-

cess resulted from the single mindedness with which he pursued anatomy; many of his talented contemporaries lost depth because of their eclectic and catholic enthusiasms; the anatomical treatises which Lind has translated generally present only a fraction of those anatomists' total outputs. His translations are lucid without being anachronistic. Where appropriate, he gives modern anatomical equivalents in parentheses, but he never tries to make these texts appear more modern than they really are. The short, introductory biographical studies of his subjects generally contain new material and full assessments of modern secondary literature.

In sum, this fine work tells us a great deal about the anatomical milieu in which Vesalius worked. It helps us see Vesalius' undoubted genius in clearer perspective. Though Professor Lind's bibliographic enthusiasm will appeal only to a handful of specialists, the work as a whole deserves a wide audience. Together with his earlier translations of Berengario and of Vesalius' *Epitome*, it permits the Latinless reader to appreciate for himself the development of anatomy in the first half of the 16th century. Translation and annotation may not be the most exciting part of scholarship, but they can be most exacting, and Professor Lind possesses the tenacity and erudition of the ideal translator. At \$18.00 his latest book is an unusual bargain at a time when book bargains are few and far between.

W. F. Bynum

Drug effects

Handbook of Drug and Chemical Stimulation of the Brain: Behavioural, Pharmacological and Physiological Aspects. By R. D. Myers. Pp. xvi+759. (Van Nostrand Reinhold: New York and London; Medical Economics: Oradell, New Jersey; February 1975.) £19.90.

TECHNIQUES for the direct administration of drugs into the cerebrospinal fluid, or into discrete areas of the central nervous system (CNS) have been in use for some time and have proved increasingly popular. The apparent directness and disarming simplicity of this approach to the examination of the CNS pharmacology of various bodily functions or behavioural processes has been particularly attractive to many research workers in the fields of physiological psychology and neuroendocrinology. This volume describes the many practical difficulties and pitfalls associated with this approach.

The main advantages of the direct administration of substances into the CNS are, first, that one may examine the effects of compounds that do not readily penetrate the blood-brain barrier after systemic administration and, second, that by localised applications one may hope to localise a particular drug action to some particular neuronal system in the CNS. The approach suffers, however,

from severe practical limitations. Unless very small volumes of fluid are injected, the spread of substances may be far too great to allow localisation. It is also difficult to judge what an appropriate dose of injected material should be. If compounds that have highly selective pharmacological actions on peripheral organs are injected in high local concentrations into the CNS, the observed effects may result from the non-specific toxic or membrane stabilising effects commonly found in many drugs. The absolute specificity of action of any drug is a myth that many of those engaged in research in this area have found beguiling.

In spite of the practical and theoretical difficulties, there is little doubt that research of this type has many useful applications. Although sophisticated methods exist for the micro-application of drugs and other substances onto single neurones in the CNS by micro-iontophoresis, one could only expect to elicit observable changes in behaviour or physiological functions after the exposure of much wider populations of neurones to drug stimulation or inhibition. Dr Myers is an acknowledged authority in this area and he has prepared a useful and scholarly compendium of the literature. He discusses in detail the principles and experimental methods in current use, and reviews the results obtained in attempts to manipulate a wide variety of bodily functions or behavioural states, including the control by the CNS of cardiovascular and respiratory functions, neuroendocrine relationships, sexual behaviour, body temperature, hunger, thirst, sleep, arousal, pain and cognitive functions. Each topic is dealt with in sufficient detail to justify the use of the term 'Handbook' in the title of the volume.

The book is amply illustrated, and each section has a "Master Table" summarising the available results. It is also liberally documented, with more than 1,400 references. Unfortunately, its value is diminished greatly by the fact that references cover the literature in detail only up to early 1972; this results in what seems to be a curiously dated view on some issues. For example, the evidence that cholinergic mechanisms play a rôle in learning and memory is reviewed in some detail, whereas the rôle of monoamines is dealt with in half a page. The latter is one area in which much has happened since 1972.

Dr Myers has presented facts and has not, to any extent, aimed to integrate psychological, pharmacological and physiological findings.

The author has produced the first detailed survey of an active and often poorly understood area. The volume will certainly be a useful reference work for those interested in this field.

S. D. Iversen

Drugs and Behaviour: A Primer in Neuropsychopharmacology. By Ernest L. Abel. Pp. ix+229. (Wiley-Interscience: New York and London, October 1974.) £7.55.

THE word 'primer' was originally used at the time of the Reformation to describe a prayer book or devotional manual for the use of the laity. Only later did the term acquire its common connotations of inky schoolroom, and the plain child's introduction to simplified truths.

A primer in neuropsychopharmacology might be expected to fulfil something of both these useful functions. The members of the laity who are not specialists in this particular science will be grateful for a reliable and intelligible guide to esoteric mysteries; and those who are in the graduate schoolroom, beginning the specialist study of this subject will be grateful for an accurate professional introduction.

At first glance this book has much to recommend it under both headings. It is eminently readable, and its author has natural gifts as a master of exposition. Illustrative examples are well chosen to exemplify general scientific principles—hunger as an adrenergic mechanism, the cholinergic mediation of water intake, serotonergic mechanisms and sexual behaviour. Headings and sub-headings order the matter well. Graphs are introduced at the apt moment, and are clear in the messages they carry. All in all, the immediate impression is of a simple textbook of considerable substance and expert presentation.

Closer reading brings some disappointments. The 'psycho' element in the neuropsychopharmacology (a truly Germanic word), receives scant treatment. The word 'placebo' is not to be found in the index, and nowhere does the influence of set and expectation receive adequate attention.

And as one gets deeper into the text so many errors and omissions come to light that one begins to feel that the book has been contrived as basis for a game of 'spot the deliberate mistake'. THC is described as "the" active material in cannabis. The picture of amphetamine psychosis remains undescribed and classical work on the matter is ignored and unreferenced. The description given of schizophrenia would make Bleuler turn in his grave, and is not only misleading but damagingly misleading. The sharp differentiation drawn between psychological and physical dependence must be described as nonsense.

In short, the author should be encouraged to produce as a second edition of this readable and essentially worthwhile book (which ought to reach many editions) a text which will neither mislead the laity nor misinform the schoolroom. Otherwise anathema, and a black mark.

Griffith Edwards

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18 Bedford Square,
London WC1B 3JN.

Generally animals . . .

Animal Physiology: Adaptation and Environment. By Knut Schmidt-Nielsen. Pp. xvi+699. (Cambridge University Press: London, March 1975.) £7.25.

No animal exists, or can exist, independently of an environment. It may logically be argued, therefore, that all physiology is environmental physiology, for physiology is concerned with the functions of living organisms. The book under review deals at an elementary level with the familiar subjects of physiology, such as respiration, circulation, digestion and so on. Its 13 chapters are grouped according to major environmental factors: oxygen; food and energy; temperature; and water. Finally, movement, information, and integration are discussed, and it is shown how these functions are correlated and controlled in the living organism. There are six appendices and a comprehensive index. Although published by the syndics of the Cambridge University Press, the book has been printed in the United States of America and uses trans-Atlantic conventions and spelling.

As in his earlier books, the Professor of Physiology at Duke University writes with a clarity that imparts beguiling simplicity to quite complex concepts. He states in the preface that his latest effort was written in "anger and frustration" because he was unable to give his students a book which, in simple words, says what he finds "exciting and important in

animal physiology, that deals with problems and their solutions, that tells how things work". He has succeeded in producing just such a book, and it will doubtless satisfy quite precisely the requirements of American undergraduate syllabuses. As far as the British market is concerned, however, I fear that the level may be a trifle too elementary.

The field of environmental physiology is now so vast that not even this large text-book can avoid being somewhat superficial. There are plenty of exciting ideas, but they are discussed too simply, and their documentation is rather inadequate. The selection of references, which "vary from brief and simple essays to large, comprehensive treatises", seems arbitrary and erratic. In places, too, there are signs of untoward haste in the writing; for example: "The degree of heating that a lizard can attain by heating up in the sun can be spectacular" (p. 358); and: "For aquatic insects the problem is to eliminate excess water and, as was mentioned above, this problem is usually handled by the kidney or equivalent excretory organ" (p. 418-9).

It is easy to cavil at the efforts of others: and the wider an author's vision, the easier it becomes to criticise his work on points of detail. There are, naturally, many aspects both of fact and of emphasis, with which other physiologists and zoologists may disagree—but this should not blind them either to the magnitude of Schmidt-Nielsen's conception or to the skill with which he has realised it. J. L. Cloudsley-Thompson

. . . particularly men

Physiology of the Human Body. By J. Robert McClintic. Pp. xxvii+588. (Wiley: New York and London, January 1975.) £6.80.

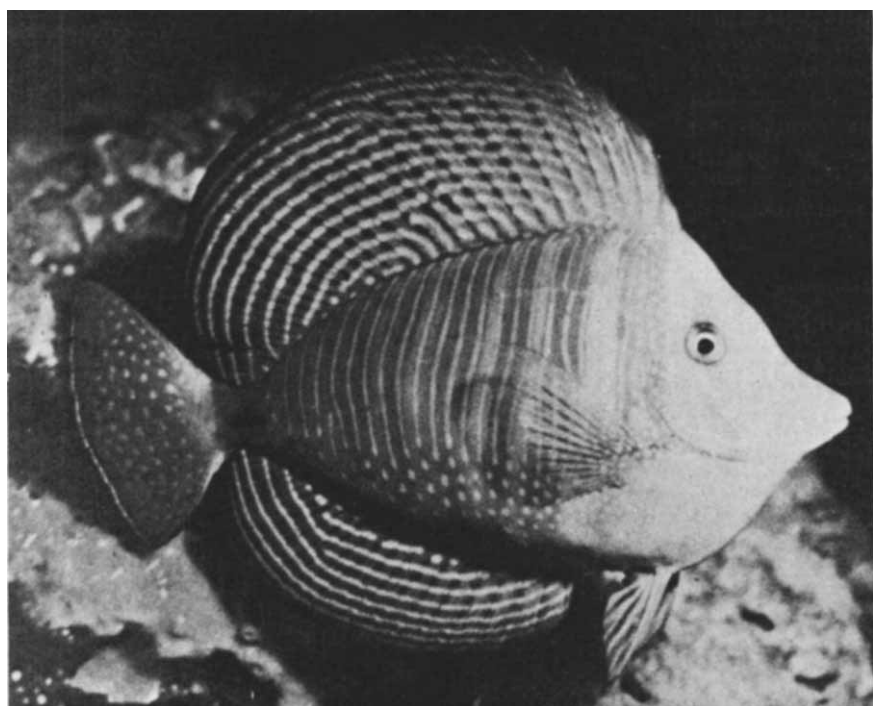
A MAJOR difficulty confronting any reviewer of this book is that of indicating clearly the type of reader to whom it should appeal, especially as the author states that it is directed at "all levels of reader" including those "planning to enter a health-orientated profession".

Among those professions, however, medicine is not specifically mentioned, and, in fact, the book is not one which could be recommended to a medical student as his working text since too many topics are treated superficially without sufficient attention to experimental evidence. The author has, however, produced a masterpiece of didactic compression which is commendably free from serious inaccuracies.

The text is liberally embellished with exceptionally clear tables and figures, often in the multicolour format familiar to readers of *Scientific American*, to which frequent reference is made in the bibliography. A most valuable feature of the book is a series of foldover plates depicting the structure of the human body layer by layer, and some might think that a study of these could save a great deal of time commonly spent in the dissecting room.

So much information is crammed into some 550 pages that redundancy is minimal and, therefore, great concentration is demanded of the reader. To aid him in monitoring his progress, however, each chapter concludes with a summary and a set of questions. Much attention is given to diseased states (presumably in the interest of "relevance") but the wisdom of including a thumb-nail sketch of the symptomatology of acute appendicitis is debatable. Likewise, an attempt to summarise biochemistry in a 12 page appendix, composed almost entirely of structural formulae, seems ill-judged, as does the inclusion of an elaborate table listing the symptoms of drug abuse and providing a key to junky slang; and the glossary at the end of the book usurps the function of the lexicographer by including such definitions as "quality—the nature and characteristic(s) of something". Misprints are rare but an eye-catching example amongst the questions on the digestive tract reads as "Discuss the importance of the lover in body function".

The growth in popularity of courses in human biology in both schools and universities may well ensure for this book a large market. R. V. Coxon



Zebrasoma, the sail-finned surgeon fish. From *The Guinness Guide to Underwater Life*. By Christian Petron and Jean-Bernard Lozet. 224 pages including over 100 colour illustrations and 280 in black and white. (Guinness Superlatives: Enfield, April 1975.) £6.95.

Mathematical tools

The Mathematics of Diffusion. Second edition. By J. Crank. Pp. viii+414. (Clarendon: Oxford; Oxford University Press: London, March 1975.) £12.50.

THIS volume could be regarded more as a tool kit than a book. Most of the content comprises a collection of mathematical methods for the analysis of diffusion problems, together with the solutions of the appropriate equations for many common situations. As mathematical results tend to be less transient than those of most other sciences, the bulk of the book is unchanged from the first (1955) edition, save for the addition of new references. In this new edition there are many cross-references to its alter-ego *The Conduction of Heat in Solids* by Carslaw and Jaeger; the latter volume presents a wider range of solutions to the diffusion equations and Crank has chosen those which are most appropriate to diffusion problems.

New material in this edition includes a chapter on non-Fickian diffusion, which provides an interesting review of solvent diffusion in glassy polymers, but it is somewhat out of place in this book in that it is inconclusive. A new chapter on diffusion in heterogeneous media is particularly timely as composite materials have become so widely used. The chapter

on numerical methods has been extensively revised to allow for the dramatic effects of developments in digital computers. Sadly, however, several diagrams of quaint analogue calculating machines have been lost in the process. This chapter is too brief, given that it is now often easier to compute a numerical solution than to derive the analytical solution, if one is not too proud. The challenge to a book of this kind is to provide enough detail to allow the average user to write the programme without consulting other sources. Crank dodges this challenge and simply gives a well referenced outline of the available methods.

This volume is a worthy successor to the first edition and will undoubtedly become an indispensable reference source for all who are concerned with diffusion problems.

P. D. Calvert and N. C. Billingham

Dynamic patterns

Dynamic Patterns of Brain Cell Assemblies. By A. K. Katchalsky, V. Rowland and R. Blumenthal. Pp. 187 + viii. (MIT Press: Cambridge, Massachusetts, and London, 1974.) \$15.00; £7.50.

WHAT is meant by "dynamic patterns"? In the present context the expression implies the idea that systems are made up of a complex hierarchy of smaller and larger "flow patterns" in which "things" are self-maintaining features of the flows. The concept also implies that the flow patterns can undergo sudden flip-over alterations, or translations, to new stable self-maintaining arrangements. Thus, one could say that the world is made up of complexes of movements, or dynamic patterns.

How important are these dynamic patterns? Are they merely biomathematicians' fantasies—intellectual toys—or are they of fundamental significance in biology? This book reports on a Neurosciences Research Programme Work Session, held in 1972, when various neuroscientists came together to consider the phenomenology of dynamic patterns and their relevance to neurobiology. Examples of dynamic patterns are presented from many fields including biology—the cellular structure that develops in a heated dish of spermacti oil, self-maintaining cloud patterns, the well known Belousov-Zhabotinsky reaction, and so on. Indeed, the invocation of dynamic patterns, or "cooperative non-equilibrium phenomena" is fashionable in fields as diverse as molecular interactions, developmental biology and sociology. My feeling is that these phenomena are likely to be of great significance, particularly for developmental biology and neurophysiology, and this book provides a useful introduction for non-specialists.

R. M. Gaze

Global tectonics and fossil fuel

Petroleum and Global Tectonics. Edited by Alfred G. Fischer and Sheldon Judson. Pp. xii+322. (Princeton University Press: Princeton and London, May 1975.) Cloth, £8.70; paper, £4.45.

At the present time the surest way to get any geological manuscript into print is to postscript the title '... and Global Tectonics'.

This volume, *Petroleum and Global Tectonics*, contains nine papers presented at a conference held in 1972 to honour Hollis D. Hedberg of Princeton University. The papers cover plate tectonics (Bullard), heat flow and vertical movements of the crust (Morgan), the origin and growth of basins (Fischer), rift valley basins and the sedimentary history of trailing continental margins (Kinsman), the petroleum and plate tectonics of the southern Red Sea (Exxon), marine sediments, geosynclines and orogeny (Curry), geochemical formation of oil (Erdman), geothermal gradients, heat flow, and hydrocarbon recovery (Klemme), and the distribution and geological characteristics of giant oil fields (Moody).

The idea of choosing a conference theme of interest to both academic and industrial geologists is highly commendable. But the academic and industrial authors seem to have been mutually embarrassed and to have been uneasy about the theme. Sir Edward Bullard thought the impact of plate tectonics on petroleum exploration was "rather weak" (p. 17), and Moody was similarly perplexed: 'I have some difficulty in trying to relate giant oil fields to the "New Global Tectonics" *per se*' (p. 314).

The net result is that most authors have gone off and 'done their own thing', either writing about global tectonics or about petroleum, but seldom about both.

It is only in the papers by the Exxon group, Klemme and Moody, that the two topics of petroleum and global tectonics are actually integrated, and very successfully so. The material for these three contributions is largely taken from back numbers of the *Oil and Gas Journal* and from the *Bulletins and Memoirs of the American Association of Petroleum Geologists*.

The volume does include, however, several excellent papers on sedimentary basins, geothermal gradients, modern marine sedimentary environments, rift valleys and the origin of oil.

The book is attractively produced and the illustrations are ample and clear.

R. C. Selley

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obituary

Constantinos Doxiadis, known for his planning projects in a number of developing countries, has died in Athens at the age of 62.

Born in Bulgaria, he studied architecture at Athens Polytechnic and obtained a doctorate in engineering at Charlottenburg Technical University, Beira. He was director of the Athens Technological Institute and founder of the World Society of Ekistics, a term, coined by him to describe the interdisciplinary science of human settlements. He promoted a unique and valuable series of conferences on town planning studies, held annually in Athens and the Greek islands during the past dozen or so summers. To these Delos symposia—probably Doxiadis' primary contribution to his field—he attracted many leading exponents of different disciplines, who would not otherwise have had the opportunity to exchange ideas. Doxiadis was, at the time of his death, preparing a number of documents for the United Nations conference on human settlement, to be held at Vancouver in 1976.

Elizabeth L. Hazen, the coinventor of Nystatin, the first antibiotic successful in combating human fungal diseases, died in Seattle at the age of 89.

The greater part of Dr Hazen's career was spent with the state Health Department's division of laboratories and research. She and her coworker there, Dr Rachel F. Brown, screened

hundreds of soil samples in the search for new antibiotics, finally tracing a microorganism that produced the antibiotic which became known as Nystatin. She received her doctorate in bacteriology from Columbia University, after graduating from the State College for Women in Columbia, Mississippi, and also served as an investigator in the department of dermatology at Columbia University College of Physicians and Surgeons.

Sir Geoffrey Ingram Taylor, a mathematician who made valuable contributions to engineering and geophysics, has died at the age of 89.

Sir Geoffrey had mainly been concerned with abstract hydrodynamical themes and later progressed to research in the practical problems of geophysics and meteorology. He was formerly Yarrow Research Professor of the Royal Society, of which he became a fellow in 1919. In 1913, he was meteorologist to the Scotia Expedition to the North Atlantic, and in France during World War I, he served as meteorologist to the RFC. He resumed this appointment during World War II and studied the motions of the air, and the cause and effect of eddies—many of these problems were applicable to aircraft. He was also involved in the design of better parachutes. He worked with the group which caused the first nuclear explosion at Los Alamos, New Mexico in 1944 and in

that year he was knighted. Later in 1944, he won the Copley Medal, the senior award of the Royal Society, for contributions to aerodynamics and the structure of metals.

Joseph Proudman, CBE, FRS, the distinguished mathematician and oceanographer, has died at the age of 86.

Professor Proudman first studied the dynamics of tides at Trinity, Cambridge, and this was to become his main scientific interest. He returned to Liverpool, where he had taken his first degree, as a lecturer in 1913, and was appointed the first professor of applied mathematics in 1919. In 1933 he transferred to the chair of oceanography, which he held until his retirement in 1954. In 1916 Horace Lamb asked Proudman to assist him in preparing a report for the British Association on the state of research on ocean tides. This led him to found the Tidal Institute (now Bidston Laboratory of the Institute of Oceanographic Studies), concerned with all aspects of tides. The Institute acquired an international reputation for its tidal prediction services as well as for fundamental research. He was pro-vice-chancellor of Liverpool from 1940–46. He was a fellow of the Royal Society, which awarded him the Hughes Medal in 1957. He acted as secretary of the International Association of Physical Oceanography and as president from 1951–54.

announcements

Award

The Canadian Public Health Association has awarded the 1975 **ORTHO Award** to **J. E. F. Hastings** for his research in health services, planning and education.

Appointments

The National Environmental Research Council has appointed **A. W. Woodland** as director of the Institute of Geological Sciences.

Michael Sela has been elected president of the Weizmann Institute of Science in Rehovet, Israel.

International meetings

September 1–2, **Molecular beam kinetics**, Heriot-Watt University, Edinburgh (Dr A. R. Burgess, Department of Chemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK).

September 1–3, **Thermodynamical properties of gravitational fields**, Newtown, mid-Wales (B. F. Schultz, Department of Applied Mathematics and Astronomy, University College, PO Box 78, Cardiff CF1 1XL, UK).

September 1–3, **Industrial crystallisation**, Usti Nad Labem, Czechoslovakia

(Dr Sci Ing Nyvlt, Research Institute of Inorganic Chemistry, Revolucni 86, CS400, 60 Usti Nad Labem, Czechoslovakia).

September 1–4, **Electronic properties of solids under high pressure**, Louvain, Belgium (Luan Gervan, Laboratories of Solid State Physics, Department of Physics, Celestunenlaan 200D, B30/0, Leuven, Belgium).

September 1–5, **Charles Lyell centenary symposium**, London (J. C. Thackeray, Institute of Geological Sciences, Exhibition Road, London SW7 2DE, UK).

September 1-5, **Low energy electron diffraction and electron spectroscopy of solids**, Budapest, Hungary (Secretariat, LEED and Electron Spectroscopy of Solids Conference, Research Institute for Technical Physics, 1325 Budapest, PO Box 76, Hungary).

September 1-5, **Luminescence**, Tokyo, Japan (S. Shionoya, Institute of Solid State Physics, Tokyo University, 7221 Roppongi, Minato-ku, Tokyo 106, Japan).

September 1-5, **Combustion**, Orleans, France (Secretariat of the second European Symposium on Combustion, CRCCCH-CNRS, Avenue de la Recherches Scientifique, CEDEX F45045, Orleans la Source, France).

September 1-5, **Computer education**, Marseille, France (AFCET, Immeuble Centre Dauphine, Place du Marechal de Lattre de Tassigny, Paris 16e, France).

September 1-5, **Controlled fusion and plasma physics**, Lausanne, Switzerland (F. Hofmann, Centre de Recherches en Physique des Plasma, Ecole Polytechnique Federale de Lausanne, Avenue des Bains 21, CH1007 Lausanne, Switzerland).

September 1-5, **Drug abuse**, London (International Council on Alcohol and Addictions, Box 140, 1001 Lausanne, Switzerland).

September 1-5, **Mechanics in reactor technology**, London (Mr H. M. Caruthers, Associated Nuclear Services, 14-16 Regent Street, London SW1Y 4PH or British Nuclear Energy Society, 1-7 Great George Street, London SW1P 3AA, UK).

September 2-4, **Quantum electronics**, Oxford (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

September 2-4, **Energy utilisation and conservation**, East Kilbride (Conference Organiser, Birniehill Institute, National Engineering Laboratory, East Kilbride, Glasgow G75 0QU, UK).

September 2-5, **Electronic circuit techniques**, Canterbury, UK (Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL, UK).

September 2-6, **Cell differentiation**, Copenhagen, Denmark (DIS Congress Services, 3 Knabrostraede, DK 1210 Copenhagen, Denmark).

September 2-6, **Neurochemistry**, Barcelona, Spain (Dr J. Sabater, Instituto de Bioquímica, Calle Roberto Bassas 1, Barcelona 14, Spain).

Person to Person

'Yeti' discovered in Bhutan



A mummy-like object found in the state of Bhutan in the Himalayas and photographed by Pushkar Singh, who believes that it is the remains of a 'yeti' or abominable snowman. He would be grateful for any comments from readers who may have come across such an object on their travels (82 Mehdiapatnam Colony, Hyderabad 500028, Andhra Pradesh, India).

Disaster message. Research group invites voluntary involvement of people from all disciplines interested in improving disaster assessment and relief including possible short term overseas surveys of up to 3 months (London Technical Group, 41 Queen's Gate, London SW7, UK, tel. no. 01-589 9076 ext. 14).

Immunodeficiency - cancer. Any physicians who have treated persons with primary immunodeficiency diseases who have subsequently developed cancer or who know of such cancer cases, are encouraged to register them in an international immunodeficiency-cancer registry. Information on patients confidential, data from the registry available to all participants (B. D. Spector or J. H. Kersey, Immunodeficiency-Cancer Registry, Box 609 Mayo, University of Minnesota, Minneapolis, Minnesota 55455).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

September 3-5, **Microtubules and microtubular inhibitors**, Beerse, Belgium (M. Borgers or M. de Brabander, Janssen Pharmaceuticals, Research Laboratories, 2340-Beerse, Belgium).

September 3-6, **Lipoproteins and hyperlipidemias**, Lisbon (Professor M. J. Halpern, Department of Biochemistry, Faculty of Medicine of Campo Santana, Lisbon, Portugal).

September 3-8, **Artificial intelligence**, Tbilisi, USSR (Professor E. Sandewall, General Chairman IJCAI-75, Artificial Intelligence Laboratory, 545 Technology Square, Cambridge, Massachusetts 02139).

September 4-9, **Stereology**, Gaithersburg, USA (Fourth International Congress for Stereology, Organising Committee, Dr G. A. Moore, National Bureau of Standards, 31203, Washington DC 20234).

September 7-9, **Conservation for decision makers**, Kinshasa, Zaire (International Union for the Conservation of Nature, 1110 Morges, Switzerland).

September 8-10, **Engineering and food quality**, Cambridge, UK (Dr R. Jowitt, National College of Food Technology, St George's Avenue, Weybridge, Surrey, UK or Gesellschaft Deutscher Chemiker - Geschäftsstelle D6000, Frankfurt, M90 Postfach 900440, German Federal Republic).

September 8-11, **Gas dynamics of explosions and reactive systems**, Orleans, France (Centre National de Recherche, Scientifique 45045, Orleans-Cedex, France).

September 8-11, **Electron microscopy and analysis**, Bristol, UK (Dr J. E. Steeds, H. H. Wills Physics Laboratory, University of Bristol, Bristol BS8 1TL, UK).

September 8-11, **Marine natural products**, Aberdeen, UK (Dr J. F. Gibson, The Chemical Society, Burlington House, London NW1V 0BN, UK).

Miscellaneous

Vision research. In celebrating their 350th anniversary, the Worshipful Company of Spectacle Makers proposes to award a limited number of research fellowships (up to £3,000 per annum for up to 3 years). Intended as personal support only—the recipient's university or place of research will be expected to provide working accommodation, apparatus and facilities.